Mapping of Executive Functions in Children and Adolescents Born Very Preterm

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Summary

Executive function deficits are among the most frequent sequela of very preterm birth but the underlying neuronal mechanisms are not yet fully understood. This thesis contributes to a better understanding by adopting a multimodal approach: Neurodevelopmental assessments were combined with quantitative magnetic resonance imaging (MRI) assessing brain structure and high-density electroencephalography (EEG) assessing brain function to map executive functions in children and adolescents born very preterm. The results show that even those individuals born very preterm with normal intellectual abilities may experience executive function deficits if the demands placed on their abilities are high. Smaller volumes of subcortical brain regions, particularly of the thalamus, were found to be related to poorer working memory abilities, one of the key executive processes. Applying high-density EEG during sleep revealed that individuals born very preterm express higher sleep slow wave activity over brain regions associated with executive processes compared to term-born peers. As sleep slow waves are thought to reflect the synchronized oscillatory activity of the thalamocortical system, these findings contribute further to the overall finding of this thesis: The thalamocortical system appears to be crucially involved in executive processes and the multimodal assessment of its structural and functional integrity provides novel insight into the long-term consequences of very preterm birth.

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Zusammenfassung

Defizite in exekutiven Funktionen gehören zu den häufigsten Folgen von Frühgeburtlichkeit, die zugrundeliegenden neuronalen Mechanismen sind bislang aber noch nicht ausreichend bekannt. Um einen Beitrag zum besseren Verständnis zu leisten bedient sich die vorgelegte Dissertation eines multimodalen Ansatzes: Entwicklungsdiagnostische Verfahren werden mit quantitativer Magnetresonanztomographie zur Erfassung der Hirnstruktur sowie hochauflösenden elektroencephalografischen (EEG)-Aufzeichnungen im Schlaf zur Erfassung der Hirnfunktion kombiniert. Es wurde gezeigt, dass frühgeborene Kinder trotz normaler kognitiver Fähigkeiten Defizite in exekutiven Funktionen aufweisen, wenn hohe Anforderungen an die Fähigkeiten gestellt werden. Ein geringes Volumen subkortikaler Hirnstrukturen, insbesondere des Thalamus, war assoziiert mit schlechten Arbeitsgedächtnisfähigkeiten, einer bedeutenden exekutiven Fähigkeit. Frühgeborene Kinder zeigten zudem in Hirnregionen, welche mit exekutiven Prozessen zusammenhängen, mehr Tiefschlafaktivität als termingeborene Kinder. Da Tiefschlafwellen die synchronisierte oszillierende Aktivität des thalamokortikalen Systems reflektieren, unterstützt dieses Ergebnis die übergeordnete Erkenntnis dieser Dissertation: Das thalamokortikale System spielt eine wichtige Rolle für exekutive Fähigkeiten und die multimodale Untersuchung seiner strukturellen sowie funktionellen Integrität kann neue Einsichten in die langfristigen Folgen von Frühgeburtlichkeit liefern.

3

Introduction

Very preterm birth is a significant risk factor for impaired brain development and later neurodevelopmental deficits. Particularly, executive function abilities are frequently impaired in today's cohorts of very preterm survivors, however, the specific underlying neuronal mechanisms have yet to be understood. The current thesis has adopted a multimodal approach employing cognitive and behavioral assessments, structural magnetic resonance imaging (MRI) and electroencephalographic (EEG) measures of brain functioning to investigate potential alterations in the structural and functional neuroanatomy of executive processes in children and adolescents born very preterm. This may provide novel insight into the long-term consequences of early disruptions to normal brain development and foster the identification of specific targets for neuroprotective agents and cognitive interventions to improve neurodevelopmental outcome following very preterm birth.

In the first part of the introduction, an overview on very preterm birth and the respective consequences for neurodevelopment will be provided. Next, methodological considerations related to the multimodal approach employed in this thesis will be introduced. Finally, the aims of the current thesis will be presented.

3.1 Very preterm birth – A risk for neurodevelopmental deficits

3.1.1 Definition, prevalence and consequences of very preterm birth

Very preterm birth describes birth before 32 weeks of gestation and occurs in 1% of all life-births (Tucker and McGuire, 2004). In Switzerland, this equals approximately 800 babies every year (BFS, 2015b). The risk factors for preterm birth are manifold and include low or high maternal age, multiple gestation, hypertensive disorders of pregnancy, intrauterine infections, intrauterine growth restriction, cervical shortening or psychological and social stress (see Tucker and McGuire, 2004 for an overview). The increase of maternal age (*e.g.* from 27.8 years in 1970 to 31.7 years in 2014 in Switzerland (BFS, 2015a)) and the increased number of multiple births, presumably due to the increase in assisted reproduction, are the main contributors of the increase in preterm births reported for most industrialized countries over the

past decades (Blondel *et al.*, 2002; Tucker and McGuire, 2004; Tandberg *et al.*, 2007). In parallel to the increased number of preterm births, advances in pre-, peri- and postnatal care have resulted in an increase of the survival rate of preterm babies, particularly of those born at the limit of viability (Moore *et al.*, 2012; Rüegger *et al.*, 2012; Schlapbach *et al.*, 2012). Together, this leads to an increasing number of very preterm infants who grow up to reach childhood, adolescence and adulthood. It is, therefore, of utmost importance to determine the prevalence, spectrum and mechanisms of neurodevelopmental sequela of very preterm birth.

Despite the decrease in mortality, the rate of very preterm survivors suffering from moderate or severe neurosensory, intellectual and motor disabilities has remained largely stable across time (Schlapbach *et al.*, 2012; see Figure 1). Approximately 10-15% of those born before 32 weeks of gestation and 20-25% of those born before 28 weeks of gestation are affected (Larroque *et al.*, 2008; Moore *et al.*, 2012; Schlapbach *et al.*, 2012). As a consequence, the proportion of children surviving without any major impairments increased across time (Rüegger *et al.*, 2012).

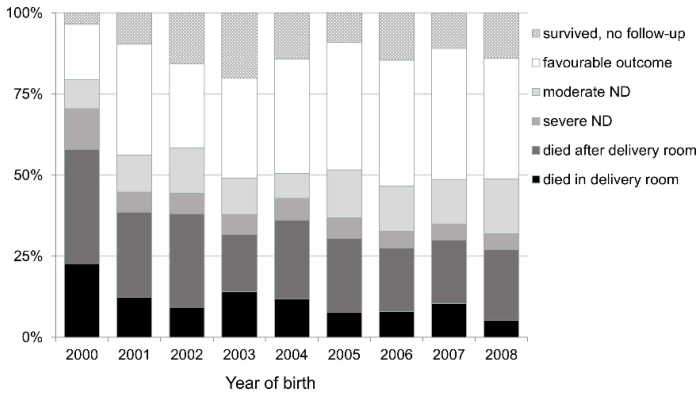

Figure 1. Change across time in outcome at two years of age after birth before 28 weeks of gestation. FU: Follow-up. ND: Neurodevelopmental disability. (adapted from Schlapbach et al., 2012).

A growing body of evidence suggests that in these children mild forms of cognitive, motor and social problems are common (see Aarnoudse *et al.*, 2009 for an overview). For example, a recent meta-analysis reported a mean reduction of the intelligence quotient (IQ) by 11.9 points in children and adolescents born very preterm compared to typically-developing term-born peers (Kerr-Wilson *et al.*, 2012). Also, attention problems (Aarnoudse *et al.*, 2009), language difficulties (Franken and Weisglas-Kuperus, 2012) and social deficits (Montagna and Nosarti, 2016) have been described. Executive function deficits have gained particular interest due to their extensive impact on child development (Anderson, 2002).

3.1.2 Executive functions and executive function deficits following very preterm birth

To date, no formal definition of 'executive functions' exists but rather, different descriptions of the concept have been suggested (see Jurado and Rosselli, 2007 for an overview). Generally, executive functions describe a collection of higher-order cognitive abilities or top-down control processes which are necessary for goal-directed and adaptive

behavior and which may be of particular importance if automatic, habitual responses are inadequate and may not lead to optimal behavioral outcome (Anderson, 2002; Jurado and Rosselli, 2007; Diamond, 2013). Abilities commonly referred to as executive functions include inhibition, interference control, working memory, mental flexibility, verbal and conceptual fluency, planning, processing speed, or conceptual reasoning (Miyake *et al.*, 2000; Anderson, 2002). To study executive functions and respective deficits, researchers typically assess a circumscribed set of abilities using commercially available test batteries (for example the Delis-Kaplan Executive Function System, D-KEFS (Delis *et al.*, 2001), the Developmental Neuropsychological Assessment, NEPSY (Brooks *et al.*, 2009) or the Cambridge Neuropsychological Test Automated Battery, CANTAB (2004, 2011)) or compilations of tailored tasks to describe executive functioning.

Executive functions have gained particular interest in recent years in developmental neuroscience as they were found to strongly impact different areas of child development: For example, in typically-developing children, executive function abilities predict school readiness (Blair, 2002) and underlie academic abilities (Bull *et al.*, 2008; Best *et al.*, 2011). Also, they play a crucial role in social interactions and are associated with quality of life (Anderson, 2002).

In children and adolescents born very preterm, executive functions deficits have been described extensively (Anderson and Doyle, 2004; Bayless and Stevenson, 2007; Nosarti *et al.*, 2007; Mulder *et al.*, 2010; Ford *et al.*, 2011; Luu *et al.*, 2011; Rose *et al.*, 2011; Aarnoudse *et al.*, 2012; Litt *et al.*, 2012; Loe *et al.*, 2012; Ritter *et al.*, 2014; Burnett *et al.*, 2015), even in children with normal early development (Ni *et al.*, 2011). While some studies report a 'catch-up' in older children (Ritter *et al.*, 2013), increasing evidence exists that executive function deficits persist into adolescence and adulthood following very preterm birth (Taylor *et al.*, 2004; Nosarti *et al.*, 2007). Importantly, these deficits have been shown to mediate the association between very preterm birth and attention problems (Mulder *et al.*, 2011; de Kieviet *et al.*, 2012a; Aarnoudse *et al.*, 2013) and academic underachievement (Mulder *et al.*, 2010; Litt *et al.*, 2012; Aarnoudse *et al.*, 2013).

As the number of very preterm survivors continues to increase and executive function deficits in the absence of any other neurodevelopmental impairments may be particularly prevalent and critical for academic achievement in these children, a better understanding of the neuronal mechanisms underlying these deficits is crucial to identify potential targets for neuroprotective agents and to develop tailored interventional approaches for this population.

3.1.3 Prenatal brain development and alterations due to very preterm birth

The structural foundations critical for neural network development are established early during gestation: Following a neurulation phase over the first four weeks post-conception, neural proliferation begins and is followed by neural migration, synaptogenesis, and apoptosis (Tau and Peterson, 2010; see Figure 2).

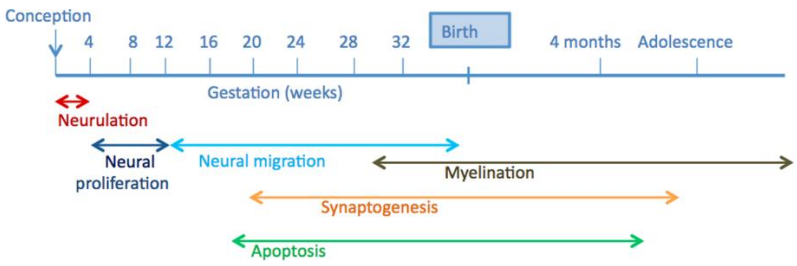

Figure 2. Timeline of major events in brain development (adapted from Tau et al., 2010)

Particularly, during the second half of gestation (*i.e.*, 20 to 40 weeks of gestation) and the neonatal period, cerebral pathways are established and the cortex undergoes rapid development (Kostović and Jovanov-Milošević, 2006; Kostović and Judaš, 2010). Very preterm birth significantly interferes with an array of neurobiological processes occurring during that time with potentially far-reaching and long-term consequences for further brain development (Volpe, 2009b; Kostović and Judaš, 2010; de Kieviet *et al.*, 2012b).

The establishment of thalamocortical connections is one of the major neurogenetic event in late fetal brain development: By mid-gestation, thalamocortical (as well as callosal and associative) afferents have grown into the transient subplate of the fetal brain and engage in synaptic interaction with subplate neurons (Kostović and Jovanov-Milošević, 2006). Two crucial tasks of the subplate neurons are 1) to serve as a functional link between these afferents and their future cortical targets, and 2) to provide pioneering axonal guidance for projections from the cortex to subcortical targets (i.e. the thalamus). This makes subplate neurons central for both cortical and thalamic development in the fetal brain (Chambers *et al.*, 1990; Volpe, 2009b). Only after 24 weeks of gestation (until approximately 32 weeks of gestation), the thalamocortical axons start invading the cortical plate of corresponding target areas in the frontal, somatosensory, visual and auditory cortex and for the first time in prenatal development, synapses form in the deep cortical plate (Kostović and Jovanov-Milošević, 2006). The presence of thalamocortical afferents and synapses in the cortical plate is the anatomical substrate for sensory input from the periphery to the cortex (Kostović and Judaš, 2010). The last weeks of fetal brain development are characterized by a gradual resolution of the transient subplate and the entrance of associative and callosal fibers into the cortex (Kostović and Judaš, 2010). Also, thalamocortical axons become more elaborated intracortically with a possible impact of sensory input on the refinement of thalamocortical circuits (Kostović and Jovanov-

Milošević, 2006). In parallel to the establishment of cerebral pathways, multiple other developmental events fall into the second half of gestation as, for example, the development of pre-myelinating oligodendrocytes and microglia peaks at this time (Khwaja and Volpe, 2008; Volpe, 2009b).

The brains of infants born very preterm undergo all of these processes while being exposed to the extra-uterine environment and adverse events associated with intensive care treatment. This puts the emerging cerebral pathways at risk for disruptions of normal development (Volpe, 2009b; Kostović and Judaš, 2010). For example, both the subplate neurons and the growing axons (as well as the pre-myelinating oligodendrocytes) are vulnerable to inflammatory processes and hypoxic-ischemic episodes which are frequent in preterm infants (Khwaja and Volpe, 2008; Kostović and Judaš, 2010). Also, the refinement of thalamocortical circuits may be affected by sensory input such as light or pain which enters the very preterm brain at an unexpected time of development (Kostović and Judaš, 2010). Consequently, birth and extra-uterine life during this crucial period of brain development may severely impact normal developmental processes of the brain and subsequently lead to persisting alterations in cortical and subcortical brain regions as well as in the integrity of cerebral networks, particularly of the thalamocortical system.

Indeed, reduced global and regional brain volume has been reported for very preterm infants (e.g., Peterson *et al.*, 2003), adolescents (see de Kieviet *et al.*, 2012b for an overview) and adults (Nosarti *et al.*, 2014) and reductions were associated with lower cognitive performance (e.g., Nosarti *et al.*, 2008; Taylor *et al.*, 2011). In addition, impaired thalamic development and disrupted structural and functional thalamocortical connectivity were found repeatedly in very preterm born infants at term-equivalent age (Boardman *et al.*, 2006; Counsell *et al.*, 2007; Srinivasan *et al.*, 2007; Smyser *et al.*, 2010; Ball *et al.*, 2012; Ball *et al.*, 2013; Toulmin *et al.*, 2015) and first evidence exists that these impairments underlie later neurodevelopmental deficits (Ball *et al.*, 2015).

Efficient executive functioning relies on widespread brain networks including frontal, parietal and subcortical regions and insults to any part of this network may impair performance (Anderson, 2002). Due to the profound impact of preterm birth on cortical and subcortical regions as well as on connecting fiber tracts (Volpe, 2009b), alterations in some parts of the executive function network are likely and may underlie executive function deficits in individuals born very preterm. Indeed, evidence exists that in children and adolescents born very preterm, smaller regional grey and white matter volumes (Nosarti *et al.*, 2008; Taylor *et al.*, 2011), reduced cortical thickness (Skranes *et al.*, 2012) and impaired white matter

microstructure (Skranes *et al.*, 2009) are associated with poor executive function performance. Also, altered neural activation patterns in executive function networks in response to tasks have been reported (Curtis *et al.*, 2006; Nosarti *et al.*, 2006; Griffiths *et al.*, 2013; Mürner-Lavanchy *et al.*, 2014).

The multimodal assessment of both the structural and functional integrity of relevant networks may help to further unravel neuronal mechanisms underlying executive function deficits in the very preterm brain.

3.2 Multimodal assessment of structural and functional neuroanatomy

Severe neonatal brain injuries and, thus, overt brain lesions are relatively rare in today's cohorts of very preterm infants (Volpe, 2003, 2009b). Rather, subtle and diffuse alterations in the structural integrity of brain networks have been reported in parallel to altered functional processing mechanisms in the very preterm brain (Ment and Constable, 2007; Nosarti and Froudist-Walsh, 2016). This complex picture of altered structural and functional brain development goes along with mild but relevant deficits in higher-order cognitive functions in the absence of severe neurodevelopmental impairments in individuals born very preterm (see Aarnoudse *et al.*, 2009 for an overview). Accordingly, the within group variability may exceed the between group differences and, thus, make the identification of specific neuronal mechanisms underlying executive function deficits in individuals born very preterm challenging (Ment and Constable, 2007). Consequently, the multimodal assessment of both the structural and functional integrity of executive function networks may be necessary for a comprehensive understanding of the alterations following very preterm birth. This thesis project employed a combination of behavioral, neuroimaging and neurophysiological tools to do so.

3.2.1 Assessing executive function abilities

As executive functions describe various different cognitive processes (see chapter 3.1.2.), typically, they are assessed with a compilation of age-appropriate performance-based (cognitive) and rating-scale (i.e., questionnaires assessing behavior) measures to allow for a comprehensive understanding of abilities and deficits (Isquith *et al.*, 2013; Toplak *et al.*, 2013).

Performance-based tests of executive functions usually aim at tapping distinct abilities which are related to specific neuronal correlates, for example a superior frontal–intraparietal network for visuo-spatial working memory (Klingberg, 2006). Assessments are typically conducted in environments which are designed to minimize distraction with the examiner guiding through the testing session and providing specific feedback or direct prompts (McAuley

et al., 2010). The results of such measures may, thus, primarily reflect processing efficiency but may only give limited insight into self-initiated structuring and organizing skills (Toplak *et al.*, 2013). Consequently, performance-based measures have been criticized for lacking ecological validity (Isquith *et al.*, 2013). In turn, rating scales aim at assessing behavioral correlates of executive function abilities as described either by an individual himself or by a proxy, for example a parent (Drechsler and Steinhausen, 2013). They may provide indicators of an individual's competence in complex, everyday problem-solving situations but may not be easily related to specific neuronal mechanism (Toplak *et al.*, 2013).

Generally, the association between performance-based and rating-scale measures of executive function abilities has been reported to be weak (McAuley *et al.*, 2010; Toplak *et al.*, 2013; Ritter *et al.*, 2014). As they appear to assess different aspects of executive functioning, it has been suggested that 'both performance-based and rating-scale measures of executive functions provide important and non-redundant information about an individual's efficiency and success in achieving goals' (Toplak *et al.*, 2013, p.138). Consequently, the current thesis project applied a comprehensive test battery including performance-based and rating-scale measures for a detailed understanding of the profile of executive function abilities and potential deficits following very preterm birth.

3.2.2 Assessing the structural neuroanatomy of executive processes

Studying patients suffering from brain lesions may provide important information on the architecture of networks supporting cognitive abilities. For example, in adults, executive function deficits have been found to be associated with lesions in different locations of a distributed network of brain regions, particularly within the frontal and parietal lobes (Barbey *et al.*, 2012). In children born very preterm, early grey and white matter pathologies were predictive of later executive function deficits (Edgin *et al.*, 2008; Clark and Woodward, 2010; Woodward *et al.*, 2011), thus, emphasizing the role of neonatal brain injury as a major risk factor for poor neurodevelopmental outcome after very preterm birth.

Another line of research has investigated how interindividual differences in brain size, particularly global and regional brain volume, is related to differences in cognitive abilities: Most studies have focused on general intelligence ('g') and overall correlations with brain volume were reported to be moderate (see Deary *et al.*, 2010 for a comprehensive overview). Also, it has been reported that more cortical grey and white matter, particularly of the frontal lobe, and cerebellar volume is associated with better executive functions (i.e., working memory) in healthy adults (Posthuma *et al.*, 2002; Posthuma *et al.*, 2003).

Following very preterm birth, global and regional brain volumes have been reported to be reduced even in the absence of overt brain lesions (de Kieviet *et al.*, 2012b), presumably as a consequence of early interruptions of normal brain development (see chapter 3.1.3). Increasing evidence exists that these reductions may underlie neurodevelopmental impairments in very preterm children, adolescents and adults (Nosarti *et al.*, 2008; Taylor *et al.*, 2011; Bjuland *et al.*, 2014). Importantly, quantitative measures of global and regional brain volume have identified structural correlates of cognitive deficits beyond of what qualitative assessments of brain injuries revealed: For example, volume reductions in the dorsolateral prefrontal cortex and sensorimotor, parieto-occipital and premotor regions at term-equivalent age were shown to predict later working memory abilities in children born very preterm, even after qualitatively assessed white matter injuries were taken into account (Woodward *et al.*, 2005).

Current cohorts of children and adolescents born very preterm predominantly show diffuse disturbances of normal development rather than manifest brain lesions. Therefore, quantitative assessments of global and regional brain volume alterations may provide important insight into neuronal correlates of cognitive deficits, particularly in executive functioning. Consequently, the current thesis project employed quantitative structural neuroimaging methods to do so.

3.2.3 Assessing functional neuroanatomy during sleep

EEG recordings allow the assessment of brain functioning non-invasively and with high time resolution (Jäncke *et al.*, 2005) and sleep provides the opportunity to do so in an unbiased way, namely when individuals are virtually disconnected from the environment (Tononi and Cirelli, 2006). Factors related to waking activity, for example attention allocation, motivation or concentration do not impact brain functioning in this state, thus, making assessments during sleep particularly beneficial when investigating neuronal substrates of motor and cognitive abilities (Lustenberger and Huber, 2012). Importantly, using equipment with multiple recording sites distributed across the scalp (see Figure 3 for an example) allows for high spatial resolution recordings and, thus, the investigation of local changes in brain functioning (Lustenberger and Huber, 2012).

Figure 3. Participant sleeping with 128-channel Electrical Geodesic Inc. Sensor Net

Sleep spindles and sleep slow waves, the two key characteristics of non-rapid-eye-movement (NREM) sleep, are of particular interest when studying the very preterm brain as

they are both depend on the activity of the thalamocortical system (Steriade *et al.*, 1993; Steriade, 2003; Crunelli *et al.*, 2015) which is at specific risk for alterations as a consequence of very preterm birth (see chapter 3.1.3). Also, they have previously been used to assess the integrity of brain networks underlying cognitive and motor abilities in typically-developing children and adolescents (Geiger *et al.*, 2011; Kurth *et al.*, 2012; Chatburn *et al.*, 2013; Pugin *et al.*, 2014; Wilhelm *et al.*, 2014).

Sleep spindles

Sleep spindles are brief (0.5 to 3 seconds) events in the sigma frequency band (10-15 Hz but see De Gennaro and Ferrara, 2003 for an overview of different definitions) reflecting periodically reoccurring rhythmic discharges of neurons throughout the thalamocortical system (Lüthi, 2013; see Figure 4 (top) for an example). As the thalamic reticular nucleus is crucially involved in their generation and their synchronization requires intact thalamocortical and corticothalamic connections (Steriade *et al.*, 1993; De Gennaro and Ferrara, 2003), sleep spindles are thought to reflect the functional integrity of the thalamocortical system as a whole.

Interestingly, in healthy individuals, sleep spindles were found to be associated with the integrity of white matter tracts, particularly of those of the corpus callosum and within and surrounding the thalamus (Piantoni *et al.*, 2013). Consequently, sleep spindles may reflect not only the functional dynamics of the connectivity at the synaptic level but also more stable structural network properties (Piantoni *et al.*, 2013).

Different approaches to characterize sleep spindles have been used previously: While some studies use the spectral power in the sigma frequency band to describe spindle activity, others detect individual spindle events either manually or with automated detection algorithms. This allows the investigation of spindle density (number of spindles per minute), the duration of individual spindles or their peak frequency. Sleep spindles have been shown to be most prominent over centro-parietal regions (Fogel and Smith, 2011) but evidence exists that the topographical distribution may be different depending on different types of spindles, namely slow (12 and 14 Hz) and fast (14-16 Hz) spindles which show a frontal and posterior predominance, respectively.

Sleep spindles were found to be strongly associated with intellectual abilities in both children and adults and were, thus, suggested to reflect the efficiency of the thalamocortical system (see Fogel and Smith, 2011 for an overview). Reports on reduced sleep spindles in patients suffering from schizophrenia, a mental disorder associated with alterations of the

thalamocortical system (Vukadinovic, 2011), further support the view that sleep spindles may be a reflection of intact and efficient thalamocortical connections.

Consequently, assessing sleep spindles and their relation to cognitive and mental functioning in typical and atypical brain development may provide valuable insight into the consequences of altered functional and structural integrity of the thalamocortical system.

Sleep slow waves and sleep slow wave activity (SWA)

Sleep slow waves apparent in the surface EEG (see Figure 4 (bottom) for an example) are high amplitude (> 75µV), low frequency (< 5 Hz) waves which result from repeated periods of synchronized firing and silence of populations of cortical neurons (Steriade *et al.*, 1993; Vyazovskiy *et al.*, 2009). Recently, the importance of the whole thalamocortical system for the full expression of physiological sleep slow waves has been stressed as thalamic input was found to be intimately involved in the generation of cortical slow waves (see Crunelli *et al.*, 2015 for an overview). Sleep slow waves have been established as markers of synaptic strengths and neural synchrony within (thalamo-)cortical networks (Tononi and Cirelli, 2006) and may, thus, be of specific interest when investigating the functional integrity of neuronal networks in typical and atypical brain development. The spectral power in the frequency range between 1 and 4.5 Hz (*i.e.*, sleep slow wave activity (SWA)) is commonly used to quantify the activity of sleep slow waves.

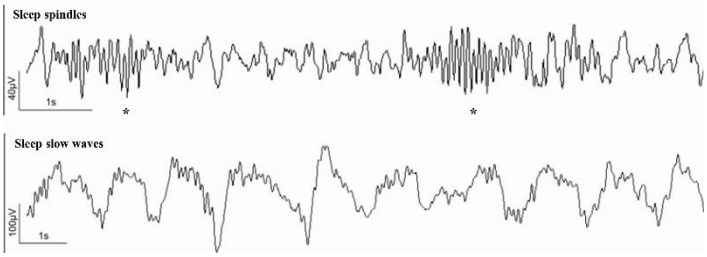

Figure 4. Main oscillations of non-rapid-eye-movement (NREM) sleep. Top: Sleep spindles (marked with an asterisk). Bottom: Sleep slow waves. Adapted from C. Lustenberger (Diss. ETH No. 21635, submitted, 2013)

Sleep SWA topography was found to reflect individual traits of functional neuroanatomy in typically-developing children and adolescents, similarly to what has previously been reported for adults (e.g., Finelli *et al.*, 2001; De Gennaro *et al.*, 2005): The topographical pattern of activity across the scalp was highly stable within an individual across multiple nights (Geiger *et al.*, 2012) even in the face of extensive maturational changes (Lustenberger *et al.*, 2016). Also, the functional neuroanatomy of cognitive and motor abilities could be reliably mapped using sleep SWA in typically-developing children (Kurth *et al.*, 2012). This raises the question of the potential feasibility of sleep SWA as a tool to investigate

altered functional neuroanatomy of motor and cognitive networks in atypical brain development, for example following very preterm birth.

Besides its potential to map the functional neuroanatomy of cognitive processes, sleep SWA may also shed light on neuroplastic processes within the respective networks as it changes locally in response to the prior use of the respective networks: For example, learning a specific motor task increased sleep SWA in the following night in brain regions known to be associated with motor learning (Huber *et al.*, 2004; Wilhelm *et al.*, 2014). Also, three weeks of intensive working memory training increased sleep SWA in brain regions known to be associated with working memory abilities (Pugin *et al.*, 2014). Sleep SWA, thus, reflects both short- and long-term use-dependent plastic changes within motor and cognitive networks and, consequently, could be of interest when assessing functional alteration and adaptation of the brain following early disruptions of typical development, for example due to very preterm birth.

In the current thesis project, comprehensive cognitive and behavioral assessments of executive function abilities were combined with the assessment of the structural and functional integrity of relevant neuronal networks to better understand the mechanisms leading to executive function deficits following very preterm birth. In the following chapter, the specific aims of the thesis will be discussed.

3.3 Aims of this thesis

The overall aim of the current thesis was to investigate neuronal mechanisms underlying executive function deficits in children and adolescents born very preterm without any major neonatal brain injuries and with normal intellectual and motor functions. They constitute the majority of today's very preterm survivors and a better understanding of the neuronal mechanisms of the deficits they frequently encounter is crucial for the development of novel interventions.

For a comprehensive understanding, we employed a multimodal approach including cognitive and behavioral assessments, structural neuroimaging and electrophysiological measures: Structural MRI has previously been used to investigate altered brain development following very preterm birth and respective consequences for different cognitive abilities and, thus, may be a feasible method to investigate the structural neuroanatomy of executive processes in children and adolescents born very preterm. In contrast, high-density EEG during sleep has not previously been used in this population. We, thus, start by showing that characteristic features of the sleep EEG reflect the integrity of the thalamocortical system and are related to cognitive and mental functioning in healthy adults. Next, we apply the findings in our clinical group to study the functional neuroanatomy of executive processes in children and adolescents born very preterm.

1) **Assessing the impact of increasing demands on executive function performance in children and adolescents born very preterm**

   Executive function deficits are frequent in children and adolescents born very preterm (see Aarnoudse *et al.*, 2009 for an overview), potentially underlying academic problems (Mulder *et al.*, 2010; Rose *et al.*, 2011; Litt *et al.*, 2012). The transition to secondary school level is accompanied by increasing demands placed on executive functions as personal autonomy, independence and planning responsibilities grow (Jacobson *et al.*, 2011; Burnett *et al.*, 2013). To date, it is unclear how children and adolescents born very preterm are impacted by increasing demands placed on their executive function abilities. Therefore, we used a comprehensive battery of tasks to systematically investigate this issue.

   Research article: *Very Preterm Adolescents Show Impaired Performance With Increasing Demands In Executive Function Tasks*. Wehrle, F., Kaufmann, L., Benz, L., Huber, R., O'Gorman, R., Latal, B. & Hagmann, C. Early Human Development (2016). 92, 37-43.

2) Assessing how global and regional brain volume is associated with working memory abilities in children and adolescents born very preterm

Global and regional brain volume has been found to be reduced in children and adolescents born very preterm (see de Kieviet *et al.*, 2012b for an overview) with the reductions being associated with impaired cognitive performance (e.g., Nosarti *et al.*, 2008; Taylor *et al.*, 2011). Working memory is a key executive function, underlying not only more complex executive abilities (*e.g.*, planning) (Miyake *et al.*, 2000; Anderson, 2002) but also predicting other abilities which may crucially impact child development (Martinussen *et al.*, 2005; Bull *et al.*, 2008; Alloway and Alloway, 2010). To date, no study has looked at potential alterations in the structural neuroanatomy of working memory abilities in school-aged children and adolescents born very preterm. Thus, we assessed global and regional brain volume using structural MRI and investigated potential relations to working memory abilities in children and adolescents born very preterm and in typically-developing term-born peers.

Research article: *Subcortical Cerebral Volume Is Associated With Working Memory Performance in Adolescents Born Very Preterm*. Wehrle, F., Buchmann, A., Guggenberger, R., Huber, Latal, B., R., O'Gorman, R., & Hagmann, C. Submitted.

3) Assessing the integrity of neuronal networks in relation to cognition and mental functioning in typical and atypical brain functioning

The integrity of the thalamocortical system has been reported to be impaired in very preterm born infants (Boardman *et al.*, 2006; Counsell *et al.*, 2007; Srinivasan *et al.*, 2007; Ball *et al.*, 2012; Ball *et al.*, 2013) with disruptions being related to poor cognitive development (Ball *et al.*, 2015). Sleep spindles and sleep slow wave activity (SWA) are the two dominant features of sleep, both depending on the activity of the thalamocortical system. We aimed to investigate how these unique electrophysiological characteristics may improve our understanding of cognitive and mental abilities in typical and atypical brain functioning.

a) How are sleep spindles related to mental functioning in healthy adults?

Sleep spindles reflect anatomical and functional differences of the thalamocortical system and have been found to be altered in patients suffering from schizophrenia, a neuropsychiatric disorder associated with impaired thalamocortical connectivity (Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010). We investigated how schizotypal personality traits in healthy individuals are related to sleep spindles to further establish

sleep spindles as a measure of thalamocortical integrity in typical and atypical brain functioning.

Research article: *Sleep Spindles Predict Schizotypal Personality Traits and Thalamic Glutamine/Glutamate in Healthy Subjects*. Lustenberger, C., O'Gorman, R., Tüshaus, L., Wehrle, F., Achermann, P. & Huber R. Schizophrenia Bulletin. 2014; 41: 522-531.

b) What characteristics of sleep spindles are related to memory consolidation in typical brain functioning?

To date, the investigation of diverse aspects of sleep spindles in isolation has limited the comprehensive understanding of how sleep spindles are related to cognition in typical brain functioning. This is, however, essential to understand how sleep spindles and potential alterations thereof are associated with cognition in atypical brain functioning, for example, following very preterm birth. Thus, we assessed spindle characteristics in a multi-dimensional manner in a group of healthy adults and related them to memory performance, a key process of cognition.

Research article: *The Multidimensional Aspects of Sleep Spindles and Their Relationship to Word-Pair Memory Consolidation*. Lustenberger, C., Wehrle, F., Tüshaus, L., Achermann, P., Huber, R. Sleep (2015). 38, 1093-1103.

c) Can sleep SWA map alterations in the functional neuroanatomy of executive processes following very preterm birth?

Sleep SWA, reflecting the synchronized oscillatory activity of the thalamocortical system, has been used to map cognitive and motor skills in typically-developing children and adolescents (Kurth *et al.*, 2012; Pugin *et al.*, 2014; Wilhelm *et al.*, 2014). We investigated whether sleep SWA can be used to map executive processes and whether alterations following very preterm birth are apparent.

Research article: *Sleep EEG Maps the Functional Neuroanatomy of Executive Processes in Adolescents Born Very Preterm*. Wehrle, F., Latal, B., O'Gorman, R., Hagmann, C., Huber, R. (submitted)

In the following chapter, the articles as listed above will be presented.

4

Research articles

4.1 Very Preterm Adolescents Show Impaired Performance With Increasing Demands In Executive Function Tasks

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Abstract

Background: Very preterm birth is often associated with executive function deficits later in life. The transition to adolescence increases personal autonomy, independence and, in parallel, the demands placed on executive functions at home and in school.

Aim: To assess the impact of increasing demands on executive function performance in very preterm children and adolescents with normal intellectual and motor functions.

Methods: Forty-one very preterm children and adolescents with normal intellectual and motor functions and 43 healthy term-born peers were assessed at a mean age of 13.0 years (SD: 1.9; range: 10.0-16.9). A comprehensive battery of performance-based executive function measures with different demand levels as well as a parent-rating questionnaire evaluating executive functions relevant for everyday life was applied. Standardized mean differences between groups of $d \geq .41$ were regarded as clinically relevant.

Results: No group differences were found at the lowest demand levels of working memory (*d* = .09), planning (*d* = -.01), cognitive flexibility (*d* = -.21) and verbal fluency (*d* = -.14) tasks, but very preterm participants scored significantly below their term-born peers in the most demanding levels (*d* = -.50, -.59, -.43 and -.55, respectively). These differences were clinically relevant. Executive functions relevant for everyday life were strongly impaired in very preterm participants, *e.g.*, global executive composite (*d* = -.66).

Conclusion: Very preterm children and adolescents with normal intellectual and motor functions are at high risk for executive function deficits that may only become apparent with increasing demands, potentially leading to academic and other deficits.

Introduction

Many very preterm children and adolescents experience behavioral, social and academic problems in the absence of major motor and cognitive disabilities (Aarnoudse *et al.*, 2009; Johnson and Marlow, 2011; Larroque *et al.*, 2011), resulting in a high need for support and special care (Johnson *et al.*, 2011; Larroque *et al.*, 2011). Executive functions, this is higher-order cognitive processes such as working memory, cognitive flexibility, verbal fluency or planning (Miyake *et al.*, 2000; Anderson, 2002) are also often impaired in very preterm children (Luciana *et al.*, 1999; Anderson and Doyle, 2004; Bayless and Stevenson, 2007; Aarnoudse *et al.*, 2012; Ritter *et al.*, 2013) and adolescents (Luu *et al.*, 2011; Litt *et al.*, 2012; Loe *et al.*, 2012; Burnett *et al.*, 2015). Importantly, these executive functions have been shown to be involved in the development of the social, behavioral and academic difficulties which very preterm children and adolescents frequently encounter (Mulder *et al.*, 2010; Rose *et al.*, 2011; de Kieviet *et al.*, 2012a; Loe *et al.*, 2012).

These difficulties in academic performance and other aspects of everyday life often increase or only become apparent as very preterm children become older and reach secondary school level (Johnson *et al.*, 2011). This transition to secondary school is accompanied by increasing demands placed on executive functions both at home and school as personal autonomy, independence and planning responsibilities grow (Jacobson *et al.*, 2011; Burnett *et al.*, 2013). It could therefore be hypothesized that the rising incidence of academic and other difficulties in very preterm children and adolescents is associated with the increased demands placed on executive functions during this age period and, thus, that very preterm children may have difficulties in coping with these increasing demands.

However, the effect of increasing demands on executive function performance in very preterm children and adolescents is not yet clear. To date, no study has systematically investigated how the level of demands placed on executive functions impacts task performance in very preterm children at the transition to adolescence. Specifically, it is not clear whether very preterm children and adolescents with normal intellectual and motor functions perform differently from their term-born peers in response to increasing demands.

Hence, the current study investigated the impact of increasing task demands on executive function performance in a group of very preterm children and adolescents with normal intellectual and motor functions by applying a comprehensive battery of executive function tasks with different demand levels in parallel with a parental questionnaire evaluating executive functioning in everyday life. The aim was to clarify whether increasing demands have a greater impact on executive function performance in those born very preterm compared to

those born at term, and whether everyday functional impairments are different between the two groups. We expected that the level of demands placed on executive function abilities has a greater impact on task performance and that everyday functional abilities are more strongly impaired in very preterm children and adolescents compared to healthy term-born peers.

Material and Methods

Participants and study procedure

Former patients of the Department of Neonatology, University Hospital Zurich, Switzerland who fulfilled the following inclusion criteria were recruited for the current study 1) born ≤ 32 weeks of gestation; 2) no evidence of cystic periventricular leukomalacia or haemorrhagic infarction on neonatal ultrasound; 3) no cerebral palsy or developmental delay (IQ < 85) at the routine follow-up consultations at the Child Development Center, University Children's Hospital Zurich, Switzerland between the ages of four and eight years, and 4) at the time of the assessment, they were between ten and sixteen years old. A total of 175 children fulfilled all inclusion criteria and were contacted by letter. Of these, 41 children and adolescents and their parents accepted the invitation to participate (23.4%). Those who participated did not differ from those who did not participate with regard to gestational age, birth weight, perinatal complications and IQ assessed at the follow-up consultation (all $p > .05$). Friends and siblings of very preterm participants and children and adolescents from local schools were recruited for the control group. Inclusion criteria were term birth (≥ 37 weeks gestation), no perinatal complications and no neurodevelopmental illnesses (e.g., attention-deficit-hyperactivity-disorder). Forty-three term-born (TB) participants were included and group-matched to the very preterm (VPT) participants with regard to gender and age at assessment.

Over the course of an afternoon, an examiner trained in cognitive testing administered various computerized and paper-pencil tests in a pseudo-randomized order. Participants were compensated with a gift certificate.

All data were collected between January and December 2013. The study was approved by the ethical committee of the Canton of Zurich, Switzerland. Written informed consent was obtained from a parent as well as from participants older than 15 years. Younger participants provided oral consent.

Measures

For VPT participants, perinatal data were collected from the hospital's medical records. Socio-economic status (SES) was estimated using a six-point scale based on maternal education and paternal occupation (Largo *et al.*, 1989). IQ was estimated with a 4-subtest combination of the *Wechsler Intelligence Scale for Children* (WISC-IV, German version (Petermann and Petermann, 2006)): Block design, vocabulary, letter-number-sequence, and symbol search. This subtest combination has been shown to correlate highly with the full version (r = .95) (Waldmann, 2008). In a subset of 63 children and adolescents (33 VPT and 32 TB participants), the pegboard subtest of the Zurich Neuromotor Assessment (ZNA (Largo *et al.*, 2007)) was applied. In this task, brass pegs are placed in twelve holes in a board and need to be inverted and replaced in turn using only one hand. Two runs per hand are timed and the average performance time (in seconds) of all runs is used as a measure of fine motor abilities.

EF measures

A variety of performance-based and rating scale measures were applied for a comprehensive understanding of executive function abilities (Isquith *et al.*, 2013).

Three executive function tasks of the *Cambridge Neuropsychological Test Automated Battery* (CANTAB (Cognition, 2004, 2011)) were randomly administered on a 12.1-inch touch screen tablet: Spatial working memory was assessed with the Spatial Working Memory (SWM) task, planning abilities were assessed with the Stockings of Cambridge (SOC) task, and cognitive flexibility was assessed with the Intra-/Extradimensional Shift (IES) task. Detailed descriptions of the tasks and outcome measures are provided in Table 1. Due to technical problems, data for one TB participant were lost for the SOC task.

Table 1. Description of CANTAB tasks and dependent variables.

Name of task	Task description	Dependent variables
SWM: Spatial Working Memory	The SWM task assesses spatial working memory performance. Participants are required to find tokens that are hidden under a number of boxes shown on the screen. The level of task demands increases from low (4-box trials) to high (8-box trials) as the number of boxes increases. If a box has been emptied from a token, it should not be revisited in the same trial. Four trials per demand level are administered. Each trial is completed when all tokens are found.	1) SWM revisit errors: number of times returned to a box in which a token had been found previously in the same trial (fewer = better)
SOC: Stockings of Cambridge	The SOC task is an adapted version of the Tower of London task and assesses spatial planning ability. Participants are required to plan and execute a set of movements in a lower row of 'stockings' in order to copy a given pattern in the upper row. The level of task demands increases with the increasing number of planning steps/moves required from low (2-move trials) to high (5-move trials). Participants are instructed to solve the trials in as few moves as possible. Two trials for the 2- and 3-move level and four trials for the 4- and 5-move level are administered. A trial is aborted if more than double the amount of the minimal moves necessary is executed without correctly copying the pattern.	1) SOC solved at all: % trials solved at all (more = better) 2) SOC perfect solution: % trials solved in the minimal amount of moves necessary (more = better) 3) planning time: time from presentation of trial until first ball is touched for perfect solves (longer = better)
IES: Intra-/Extradimensional Shift	The IES task is an adapted version of the Wisconsin Card Sorting Task and assesses cognitive flexibility. Two pictures consisting of different perceptual dimensions (white lines and purple shapes) are presented on the screen simultaneously. Participants are instructed to choose the one picture they believe is in accordance with a currently valid rule. They receive feedback on whether their choice is correct and therefore by trial and error discover the valid rule. After six consecutive correct choices according to the rule, the rule changes without prior notice. Participants are required to adapt their choices and identify the newly valid rule using the feedback given. In the low task demand level, an intra-dimensional shift is necessary (i.e., within the same perceptual dimension), in the high task demand level, an extradimensional shift (i.e., between the different perceptual dimensions) is necessary to adapt to the new rule. The task consists of nine shifts and is aborted if the new rule is not identified within 50 trials.	1) IDS errors: number of errors in intra-dimensional shift trials (fewer = better) 2) EDS errors: number of errors in extra-dimensional shift trials (fewer = better)

Verbal fluency was assessed using the *Regensburger Wortflüssigkeits-Test* (RWT (Aschenbrenner *et al.*, 2000)), a German-language verbal fluency test which requires participants to produce words in accordance with specific rules: In the phonetic and semantic fluency subtests, words starting with the letter 'S' or types of animals have to be generated, respectively. In the category-switching subtests, words starting with the letters 'G' and 'R' (phonetic switching) or types of fruits and sports (semantic switching) have to be named in an alternating manner. Verbal responses were recorded and later transcribed by the examiner. An additional minute of testing was added to the standard test length of one minute as the increased assessment duration of two minutes has been shown to place higher demands on verbal fluency abilities compared to one minute (Aschenbrenner *et al.*, 2000; Holtzer *et al.*, 2009). The number of words produced in the first (low demands) and the second minute (high demands) were used as dependent variables. One TB participant was not tested as German was not his first language. Data for another TB participant are missing for two subtests due to a failure of the recording device. For two VPT and two TB participants, performance was not separated in the first and second minutes, thus, only the total number of words produced within two minutes was available.

The *Behavior Rating Inventory of Executive Function* (BRIEF, German version (Drechsler and Steinhausen, 2013)) was used to investigate whether executive function deficits translate into daily life. In this questionnaire, parents rate 86 statements regarding their children's executive functions relevant to everyday life. Eight individual subscales may be summarized in a behavioral and a cognitive regulation index. Taken together, the two index scales form the global executive composite. The individual subscales, the indices, and the composite score were used as dependent variables. For all but one VPT participant, at least one parent completed the questionnaire. If both parents completed a questionnaire separately (n = 45), mean values were used for the analyses.

Statistical analyses

To compare groups, univariate analyses of covariance (ANCOVAs) with SES and age at assessment as covariates were applied for all continuous variables. To assess whether performance differences between groups were similar across age, the interaction term 'birth status x age at assessment' was also examined. Only significant interactions are reported and were retained in the final models. If appropriate, mixed-model ANCOVAs with birth status (VPT vs. TB) as between-subjects factor, task demand level as within-subject factor and SES and age at assessment as covariates were applied. To assess whether the impact of demand level

on performance was similar across age in both groups, the interaction term 'task demand level x birth status x age at assessment' was assessed. As for the univariate ANCOVAs, only significant interactions were retained in the final models. Where the assumption of sphericity was violated, Greenhouse-Geyser-corrected results are reported. Univariate ANCOVAs with SES and age at assessment as covariates were applied to follow-up mixed-model analyses.

Covariates were mean-centered before being included into the analyses. Raw scores were used to analyze the computerized performance-based tasks and the verbal fluency task. For the IQ estimate and the BRIEF questionnaire, raw scores were transformed into age-norm referenced values according to the test manuals. Standardized mean differences (SMD) given by the difference between two group means divided by the pooled standard deviation of the two groups were calculated to analyze effect sizes (*d* (Cohen, 1992)). The pooled standard deviation took into account the residuals of univariate ANCOVA models with SES as a covariate and the correlation between SES and the respective dependent variable to allow adjustment for SES (Lipsey and Wilson, 2001). Negative values indicate lower performance in VPT participants. In accordance with relevant guidelines, SMD with $d \geq .41$ are regarded as clinically relevant, as they exceed the recommended minimum effect size representing a practically significant effect, *i.e.*, exceeding small effects (Ferguson, 2009). All analyses were performed with SPSS 20.0 (SPSS, Chicago, IL, USA). The significance level was set at $p \leq .05$ (two-tailed).

Results

Demographic, socio-economic and perinatal data are summarized in Table 2. VPT participants had significantly lower estimated IQ scores (*M* [*SD*]) than TB participants (104.4 [7.3] vs. 109.5 [6.8], $p = .02$). All participants scored within the average IQ range (VPT: 91-118; TB: 99-129). Fine motor performance was not significantly different between the two groups (VPT: 22.62 [4.7] vs. TB: 22.20 [3.6] seconds, $p = .54$).

Table 2. Demographic, socio-economic and perinatal data.

	n	VPT participants	*n*	TB participants	*p*
Demographic and socio-economic data					
Age, *M* (*SD*), range (in years)	41	12.9 (1.7), 10.4 - 16.6	43	13.1 (2.0), 10.0 - 16.9	.54
Gender, male/female	41	23/18	43	21/22	.51
SES[a], *M* (*SD*), range	40	2.4 (0.9), 1-4	42	2.0 (0.9), 1-4	.03
Perinatal data					
Gestational age, *M* (*SD*), range (in weeks)	41	29.6 (2.1), 25.1 - 32.0	43	≥ 37	-
Birthweight, *M* (*SD*), range (in grams)	41	1312 (362), 840 - 1990	43	≥ 2500	-
SGA, *n* (%)	41	3 (7.3)			-
BPD, *n* (%)	41	6 (14.6)			-
NEC, *n* (%)	41	3 (7.3)			-
ROP ≥ grade 3, *n* (%)	37	0 (0)			-
PDA, *n* (%)	41	17 (41.5)		na[b]	-
Sepsis, *n* (%)	41	8 (19.5)			-
Chorioamnionitis, *n* (%)	41	7 (17.1)			-
Brain injury[c]	41				
no brain injuries, *n* (%)		30 (73.2)			-
IVH Grade I or II, *n* (%)		11 (26.8)			-

VPT: very preterm; TB: term-born; M: Mean; SD: Standard deviation; SES: socio-economic status; SGA: small for gestational age; BPD: Bronchopulminary dysplasia; NEC: Necrotizing enterocolitis; ROP: Retinopathy of prematurity; PDA: Patent ductus arteriosus; IVH: Intraventricular Hemorrhage. [a]1 = highest SES, 6 = lowest SES [b]parents reported no perinatal complications [c]brain injuries seen on neonatal ultrasound.

Performance-based executive function measures

For the CANTAB SWM task, the total number of revisit errors was not significantly different between the groups ($F(1, 80) = 2.049$, $p = .16$). The mixed-model ANCOVA revealed a significant interaction between birth status and task demand level ($F(1.338,107.016) = 4.299$, $p = .02$). Follow-up ANCOVAs revealed a significant group difference in the 8-box-trials, with the group difference exceeding the threshold for clinically relevant effects (Figure 1a, Table 3).

For the SOC task, the percentage of tasks solved at all was not significantly different between the groups ($F(1, 79) = 1.193$, $p = .28$). The mixed-model ANCOVA showed no significant interaction between birth status and task demand level ($F(1..587, 125.410) = .372$, $p = .64$). The percentage of tasks with a perfect solution was significantly lower in VPT participants ($F(1, 79) = 4.745$, $p = .03$). A significant interaction between birth status and task demand level was found ($F(3, 237) = 3.729$, $p = .01$). Follow-up ANCOVAs revealed no significant group differences in the 2- and 3-move trials but significantly lower performance in VPT participants in the 4- and 5- move trials, exceeding the threshold for clinically relevant effects (Figure 1b, Table 3). The initial planning time for perfect solves was significantly shorter in VPT participants ($F(1, 77) = 4.838$, $p = .03$). A significant interaction between birth status and task demand level was found ($F(1.620, 124.754) = 5.652$, $p = .008$). While follow-up

ANCOVAs did not reveal any significant group differences in the 2-, 3- and 4-move trials, VPT participants spent significantly less planning time in the 5-move trials, exceeding the threshold for clinically relevant effects (Figure 1c, Table 3).

For the IES task, the mixed-model ANCOVA revealed no significant interaction effect ($F(1, 80) = 3.150$, $p = .08$). Although exploratory follow-up ANCOVAs did not reveal significant group differences in either the intra- or extradimensional shift trials, the group difference exceeded the threshold for clinically relevant effects in the extradimensional shift trials (Figure 1d, Table 3).

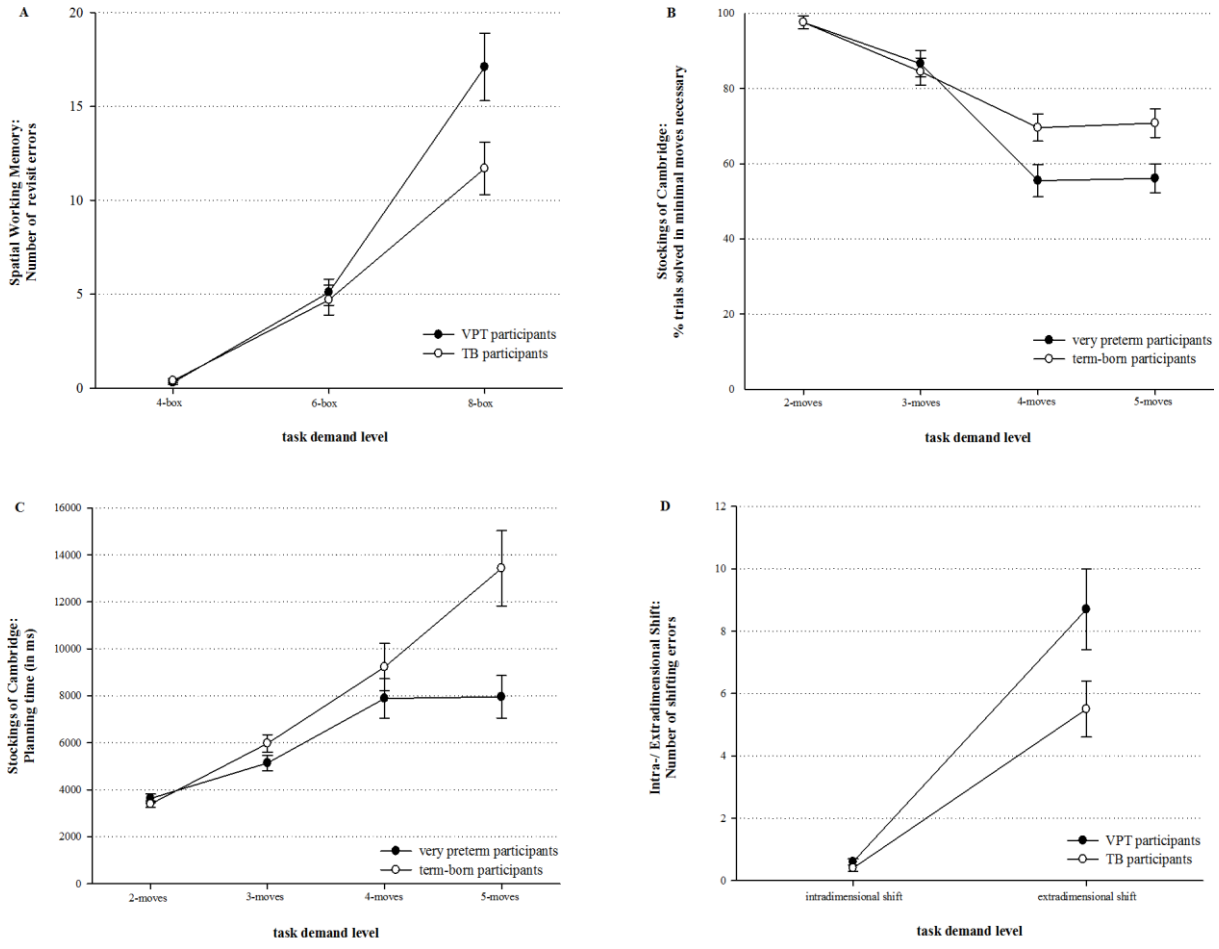

Figure 1. Performance by birth status and task demand level for all CANTAB measures. Error bars reflect ± 1 standard error.

For the phonetic switching subtest of the RWT, the mixed-model ANCOVA revealed a significant interaction between birth status and task demand level ($F(1, 75) = 4.282$, $p = .04$). Follow-up analyses revealed no significant group difference in the first minute but VPT participants produced significantly fewer words in the second minute, with the group difference exceeding the threshold for clinically relevant deficits (Table 3). No significant interaction between birth status and task demand level was found for the phonetic and semantic fluency or the semantic switching subtest. Exploratory follow-up analysis revealed that VPT participants produced significantly fewer words compared to TB participants in the second minute of the semantic switching subtest, with the group difference exceeding the threshold for clinically relevant deficits. In the semantic fluency subtest, the group difference exceeded the threshold for clinically relevant deficits in the second minute without reaching statistical significance. No difference between VPT and TB participants was found for the phonetic fluency subtest (Table 3).

Parent-rated executive function abilities

Results from the BRIEF questionnaire revealed that executive functions relevant for everyday life were significantly worse in VPT compared to TB participants for the global executive composite as well as the behavioral and the cognitive regulation index, with all group differences exceeding the threshold for clinically relevant effects (Table 3). Also, for the subscales 'emotional control' ($d = -.60$), 'initiate' ($d = -.62$), 'working memory' ($d = -.76$), 'plan/organize' ($d = -.63$), and 'monitor' ($d = -.52$), VPT participants had significantly higher T-values compared to their TB peers (group differences exceeding the threshold for clinically relevant effects; all $p \leq .05$). The subscales 'inhibit', 'shift', and 'organization of material' were not significantly different between the groups ($p > .05$), however the subscale 'shift' exceeded the threshold for clinically relevant effects ($d = -.44$).

All clinically relevant results remained significant after excluding the three VPT participants for whom parents had reported a diagnosis of ADHD (data not shown).

Table 3. Sample size, mean, standard deviation, *p*-value and standardized mean difference with respective confidence interval of all executive function measures.

	VPT participants			TB participants			p^a	d^b (95% CI)	
	n	M (SD)		n	M (SD)				
Spatial working memory - SWM									
revisit errors (total)	41	22.5	(15.3)	43	16.7	(13.3)	.16	-.39	(-.83 to .04)
revisit errors (4-box-trials)	41	0.3	(0.7)	43	0.4	(0.6)	.43	.09	(-.34 to .52)
revisit errors (6-box-trials)	41	5.1	(4.6)	43	4.7	(4.9)	.94	-.09	(-.52 to .34)
revisit errors (8-box-trials)	41	17.1	(11.6)	43	11.7	(9.3)	.05	**-.50**	**(-.93 to -.07)**
Planning - SOC									
% trials solved at all (total)	41	90.0	(8.8)	42	92.1	(6.4)	.28	-.26	(-.69 to .17)
% trials with perfect solution (total)	41	67.9	(17.4)	42	77.2	(14.7)	.03	**-.56**	**(-1.00 to -.12)**
% trials with perfect solution (2-moves)	41	97.5	(10.9)	42	97.6	(10.8)	.94	-.01	(-.44 to .43)
% trials with perfect solution (3-moves)	41	86.6	(22.4)	42	84.5	(23.4)	.40	.09	(-.34 to .52)
% trials with perfect solution (4-moves)	41	55.5	(27.7)	42	69.6	(23.1)	.03	**-.54**	**(-.98 to -.11)**
% trials with perfect solution (5-moves)	41	56.1	(24.2)	42	70.8	(24.6)	.02	**-.59**	**(-1.03 to -.15)**
planning time for perfect solutions (total, in ms)	39	6108	(2936)	42	8004	(4114)	.03	**-.52**	**(-.96 to -.08)**
planning time for perfect solutions (2-moves)	41	3630	(1215)	42	3391	(890)	.36	.22	(-.21 to .65)
planning time for perfect solutions (3-moves)	41	5135	(2128)	42	5971	(2426)	.10	-.36	(-.79 to .07)
planning time for perfect solutions (4-moves)[c]	40	7887	(5334)	42	9225	(6518)	.41	-.22	(-.66 to .21)
planning time for perfect solutions (5-moves)[c]	40	7953	(5739)	42	13'429	(10'404)	.007	**-.64**	**(-1.08 to -.19)**
Cognitive flexibility - IES									
intradimensional shift errors	41	0.6	(0.8)	43	0.4	(0.7)	.52	-.21	(-.63 to .22)
extradimensional shift errors	41	8.7	(8.6)	43	5.5	(6.2)	.06	**-.43**	**(-.86 to .00)**
Verbal fluency - RWT									
Phonetic fluency									
S-words (first minute)	39	10.3	(3.6)	40	11.2	(4.0)	.28	-.23	(-.68 to .21)
S-words (second minute)	39	6.1	(3.6)	40	7.3	(3.4)	.14	-.34	(-.78 to .11)
Semantic fluency									
animals (first minute)	39	20.4	(5.2)	39	22.2	(6.0)	.32	-.32	(-.76 to .13)
animals (second minute)	39	10.6	(4.7)	39	12.6	(3.9)	.15	**-.45**	**(-.90 to .00)**
Phonetic switching									
G-/R-words (first minute)	39	10.3	(3.0)	40	10.8	(3.9)	.77	-.14	(-.58 to .30)
G-/R-words (second minute)	39	5.2	(2.7)	40	7.0	(3.7)	.01	**-.55**	**(-1.0 to -.10)**
Semantic switching									
sports/fruits (first minute)	39	11.8	(2.9)	39	12.7	(3.5)	.34	-.27	(-.72 to .17)
sports/fruits (second minute)	39	4.6	(2.5)	39	6.2	(2.3)	.004	**-.66**	**(-1.1 to -.20)**
Everyday functioning – BRIEF[d]									
Global Executive Composite	40	52.1	(11.1)	43	46.1	(5.9)	.02	**-.66**	**(-1.10 to -.21)**
Behavioral Regulation Index	40	51.1	(9.6)	43	46.3	(6.3)	.04	**-.57**	**(-1.01 to -.13)**
Cognitive Regulation Index	40	53.1	(12.3)	43	46.5	(6.2)	.02	**-.67**	**(-1.11 to -.23)**

BRIEF: Behavior Rating Inventory of Executive Function; CANTAB: Cambridge Neuropsychological Test Automated Battery; CI: Confidence interval; IES: Intra-/Extradimensional Shift; M: Mean; RWT: Regensburger Wortflüssigkeits-Test; SD: Standard deviation; SOC: Stockings of Cambridge; SWM: Spatial working memory; TB: Term-born; VPT: Very preterm. [a]*p*-values adjusted for SES and age at assessment. [b]*d* represents the standardized mean difference (SMD) adjusted for SES, negative values indicate worse performance in VPT participants; $d \geq .41$ is regarded as clinically significant (indicated in bold). [c]planning time was not calculated for 2 participants with no perfect solutions in at least one level. [d]higher values indicate more problems.

Discussion

This study assessed the impact of task demands on executive function performance in very preterm children in late childhood and adolescence. The findings demonstrate that even very preterm children with normal intellectual and motor functions are at high risk for executive function deficits if task demands are high, as they display progressively poorer executive function performance as task demands increase. Across all CANTAB and verbal fluency tasks, clinically relevant group differences favoring term-born participants were found on trials requiring more planning (SOC), incorporating a higher working memory load (SWM), requiring extradimensional relative to intradimensional shifts (IES), and consisting of two minutes relative to one minute of word production (RWT). In addition, the deficits in executive functions relevant for everyday life were particularly evident, with effect sizes well above the threshold for clinically relevant effects.

Notably, executive dysfunctions in very preterm children and adolescents became most apparent on rating-based measures and were more subtle on performance-based psychometric tests, with group differences only becoming significant and clinically relevant when the task demands were high. This observation is consistent with previous findings suggesting that performance-based and rating-scale measures of executive functions correlate moderately (Toplak *et al.*, 2013; Ritter *et al.*, 2014) and, thus, may measure different facets of executive functions. Rating scale measures (*e.g.*, BRIEF) have been found to be sensitive to subtle deficits in executive functions relevant to everyday life, while performance-based measures reflect processing efficiency of the respective cognitive abilities in well-structured one-on-one testing sessions (Toplak *et al.*, 2013). The impairments revealed by the BRIEF questionnaire in this study may reflect the fact that everyday-life situations often consists of multiple distractive stimuli requiring different executive functions to be engaged simultaneously to cope with environmental demands (Ritter *et al.*, 2014) and, thus, every-day life may be the most demanding context for executive function abilities.

The findings of the current study reveal deficits in many different executive functions in very preterm children and adolescents. This result is consistent with findings from other studies which have identified deficits in a broad set of executive functions in this population by applying both performance-based and rating-scale measures (Luciana *et al.*, 1999; Anderson and Doyle, 2004; Bayless and Stevenson, 2007; Luu *et al.*, 2011; Aarnoudse *et al.*, 2012; Litt *et al.*, 2012; Loe *et al.*, 2012; Ritter *et al.*, 2013; Burnett *et al.*, 2015). The current study is, however, the first to demonstrate comprehensively the impairing effect of increasing task demands on performance in very preterm children and adolescents for a broad set of executive functions. Previously, similar results have been reported for planning and working memory in

very preterm children between seven and nine years of age (Luciana *et al.*, 1999) and for adolescents born before 36 weeks of gestation (Loe *et al.*, 2012). More generally, Jaekel and colleagues (Jaekel *et al.*, 2013) found that the performance of children born across the whole spectrum of GA was lower in tasks of high cognitive workload relative to tasks of low cognitive workload, with a disproportionate performance decrease in children born before 33 weeks of gestation. Importantly, in the current study, poorer executive function performance was found with increasing task demands in very preterm children and adolescents by investigating the impact of demand levels within a certain task.

Functional magnetic resonance imaging studies have reported altered activation patterns in neuronal networks underlying specific executive functions in very preterm children, adolescents, and adults, with these alterations only emerging if the task demands were high (Griffiths *et al.*, 2013; Daamen *et al.*, 2014; Kalpakidou *et al.*, 2014). This suggests that altered brain development after very preterm birth (Volpe, 2009b) may lead to distinct neuronal processing and subsequent impaired performance.

The transition from childhood to adolescence coincides with the transition from elementary to middle school (Jacobson *et al.*, 2011). Personal autonomy, independence and individual planning responsibilities increase markedly both outside (Burnett *et al.*, 2013) and within the school setting (Jacobson *et al.*, 2011). Both Luciana and colleagues (Luciana *et al.*, 1999) and Bayless and colleagues (Bayless and Stevenson, 2007) argue that parents and teachers of very preterm children may only become aware of executive dysfunctions when children have entered middle school, and thus, when the task and environmental demands increase. This age- and skill-associated increase in demands placed on executive functions may explain the widening gap in academic performance between very preterm and term-born children as they progress through elementary, middle, and high school (Allen *et al.*, 2011).

Limitations

Due to its cross-sectional design, this study cannot infer a causal relationship between executive function deficits and subsequent difficulties in coping with school and other demands. However, the aim of this study was to investigate how increasing task demands affect executive function performance in very preterm children and adolescents with normal intellectual and motor abilities. Future studies should investigate more comprehensively how these children are affected by the increasing environmental demands and what role executive function deficits play in this regard.

The sample size of this clinical study was relatively small. The data on neurodevelopmental outcome reported here was assessed within the scope of a multimodal imaging and neurophysiological study investigating neuronal mechanisms underlying executive functioning after very preterm birth. Considering the very demanding study protocol, the participation rate was within the expected range. Still, the limited sample size may have prevented several group differences from reaching statistical significance despite being of moderate size. However, according to current guidelines (Ferguson, 2009), effect sizes are interpretable even in the absence of statistical significance, and thus, we are confident that the executive dysfunctions identified in the current study not only reliably reflect the difficulties of very preterm children and adolescents in executive functions but are also of utmost relevance for clinical practice and everyday life.

Because our study sample consisted of well-functioning very preterm children and adolescents with normal intellectual and motor abilities and without severe brain injuries, our findings are rather robust. The group differences in executive function performance may have been even more pronounced if children with mild deficits in intellectual abilities (i.e., IQ 70 to 85) had been included. This selection may also have led to a ceiling effect in the lowest demand levels of the SOC and the SWM task: As depicted in Figure 1, group differences (and more performance variability) emerged only at the more demanding task levels.

The current study sample had a rather wide age range (associated with larger performance variability) as we aimed to cover late childhood as well as adolescence, important developmental periods in the context of executive functions (Burnett *et al.*, 2013). Importantly, the greater impact of task demands on performance in very preterm children and adolescents compared to their term-born peers was similar across age, as confirmed by the absence of any significant interaction effect between task demand level, birth status and age at assessment. This suggests that the impairing effect of task demands on executive function performance is consistent across late childhood and adolescence in those born very preterm.

Conclusion

Results from this study demonstrate that even very preterm children and adolescents with normal intellectual and motor abilities experience difficulties in executive functions when the demands placed on their abilities grow. In the context of the increasing demands of the school and home environments in this developmental period, careful monitoring of very preterm children beyond the transition to adolescence seems appropriate to ensure the prompt identification of difficulties and the provision of individual and tailored support where needed.

Specific training programs which take into account the level of demands may benefit very children and adolescents, improving their abilities to cope with increasing demands (Løhaugen *et al.*, 2011; Pascoe *et al.*, 2013). Eventually, targeted support and training programs may diminish the developmental gap between very preterm children and adolescents and their term-born peers.

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4.2 Subcortical Cerebral Volume Is Associated With Working Memory Performance in Adolescents Born Very Preterm

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Submitted.

Abstract

Objective Prematurity is a risk factor for later working memory deficits, also in the absence of neurodevelopmental impairments. This study aims to identify neurostructural correlates of these deficits in a cohort of school-aged children and adolescents born very preterm.

Study design Thirty-four children and adolescents born very preterm (age at assessment: *M*: 12.8 [*SD*: 1.6, *range*: 10.4-16.6] years) with normal intellectual and motor abilities were assessed. A group of 40 typically developing term-born peers was recruited (age at assessment: 13.1 [2.1, 10.0 - 16.9] years). Three-dimensional T1-weighted MR images were acquired on a 3T GE scanner and regional brain volumes were calculated using FreeSurfer. Verbal and spatial working memory abilities were assessed. Hierarchical multiple regression models and analysis of covariance were used for data analysis.

Results In the very preterm group, smaller thalamic (β = -.952) and smaller cerebellar white matter volumes (β = -.632) predicted poorer working memory performance after controlling for socio-economic status, sex, age at assessment and total intracranial volume ($F(14, 19) = 2.486$, $p = .03$, adjusted $R^2 = .38$). In term-born participants, no similar associations were found. Also, volumetric differences between low and high working memory performers were not equal in the two groups with low performing very preterm participants having smaller hippocampal and thalamic volumes compared to high performing very preterm and both low and high performing term-born peers.

Conclusion Smaller volumes in the thalamus, the hippocampus and the cerebellum, may be relevant structural correlates of the frequent working memory deficits found in very preterm survivors.

Introduction

Deficits in higher-order cognitive functions in the absence of severe intellectual or motor problems frequently affect children and adolescents born very preterm (Aarnoudse *et al.*, 2009). Working memory, the ability to temporarily maintain and simultaneously manipulate information (Baddeley, 1992), is particularly impaired (Anderson and Doyle, 2004; Saavalainen *et al.*, 2007; Luu *et al.*, 2011; Aarnoudse *et al.*, 2012; Griffiths *et al.*, 2013; Wehrle *et al.*, 2016) and such deficits have been found to mediate the association between preterm birth and attention problems (Mulder *et al.*, 2011) as well as academic underachievement (Rose *et al.*, 2011).

Studies in typically developing children and adolescents have identified a neuronal network responsible for working memory processes that encompasses fronto-parietal cortical regions but also subcortical areas such as the basal ganglia, the hippocampus, the thalamus and the cerebellum (Scherf *et al.*, 2006; Finn *et al.*, 2010; Ullman *et al.*, 2014; Darki and Klingberg, 2015). Altered structural brain development following very preterm birth has been reported to underlie impaired working memory abilities in very preterm toddlers and young children as well as young adult (see Nosarti and Froudist-Walsh, 2016 for an overview). To date, studies identifying relevant structural correlates of working memory deficits in school-aged children and adolescents are lacking. As the increase in working memory capacity is an important part of cognitive development during childhood and adolescence (Darki and Klingberg, 2015), a better understanding of the structural correlates of working memory abilities at this age may help to improve the understanding of the mechanisms leading to the deficits after very preterm birth.

Furthermore, neuronal processing in working memory networks has been found to depend on the performance level of children born very preterm with high performers showing similar processing compared to term-born peers while neuronal processing was altered in low performers (Mürner-Lavanchy *et al.*, 2014). It is unclear, however, whether structural differences exist between high and low working memory performers and how this is related to preterm birth.

Hence, the aim of the current study was 1) to identify potential structural correlates of working memory deficits in a cohort of school-aged children and adolescents born very preterm with normal IQ and motor functions, that is in individuals representing the majority of today's very preterm survivors and 2) to investigate structural differences between high and low working memory performers and its relation to preterm birth.

Materials and methods

Participants and study procedure

The eligibility criteria and the selection process for participation in the current study have been described in detail previously (Wehrle *et al.*, 2016). Forty-one children and adolescents born very preterm (< 32 weeks of gestation) without any major neonatal brain injuries and with a normal intellectual and motor development participated in the current study. They were assessed when they were between 10 and 16 years old. For the control group, 43 typically developing term-born peers were recruited. They were group-matched to the very preterm participants with regard to sex and age at assessment. All data were collected between January and December 2013 at the University Children's Hospital Zurich. The study was approved by the local ethical committee. Written informed consent was obtained from a parent as well as from participants older than 15 years. Younger participants provided oral consent.

Measures

Neurodevelopmental assessment

All participants underwent a comprehensive neurodevelopmental assessment performed by one examiner (FW). Detailed results for cognitive and motor development in this cohort have been reported previously (Wehrle *et al.*, 2016).

Spatial working memory was assessed with the Spatial Working Memory task of the Cambridge Neuropsychological Test Automated Battery (Cognition, 2004, 2011). This computerized self-ordered search task requires participants to find tokens which are hidden under an increasing number of boxes (namely, 4-, 6- and 8-box trials). Fewer errors (i.e., fewer returns to previously emptied boxes) indicate better spatial working memory abilities. Raw scores were used for further analyses as no age-normed referenced values are available for this task.

Verbal working memory was assessed with the Letter-Number-Sequencing task of the Wechsler Intelligence Scale for Children, 4th edition (Petermann and Petermann, 2006). The total number of correct trials was calculated. Higher scores indicate better verbal working memory abilities. Consistent with the spatial working memory task, raw scores were used for further analyses.

MR imaging

Image acquisition

Brain magnetic resonance imaging (MRI) was performed on a 3T GE whole-body system. Anatomical images of the entire brain were obtained with a high-resolution three-dimensional T1-weighted spoiled gradient-recalled echo sequence with repetition time = 11ms, echo time = 5ms, inversion time = 600ms, flip angle 8°. All images were anatomically evaluated by an experienced neuroradiologist (RG).

Image quality and conventional MRI

One very preterm participant refused scanning at the day of testing. One very preterm participant was excluded for further analyses due to an arachnoid cyst and subsequent left cerebellar hypoplasia. Two very preterm participants showed mild dilatation of the lateral ventricles, this was, however, not of clinical significance and participants were retained for further analyses. No other brain abnormalities were seen on conventional images.

For five very preterm and three term-born participants, the quality of the MR images was too poor for quantitative analyses. Reasons for poor quality were artefacts caused by dental braces or excessive movement. Thus, for 34 very preterm and 40 term-born participants, complete data sets were available and included for further analyses.

Image analysis

All images were transformed into Analyze format and entered into a standard morphometric analysis using Freesurfer version 5.3.0 for Macintosh computers, which is freely available online (http://surfer.nmr.mgh.harvard.edu/). This software allows the correction for motion and the removal of non-brain tissue with an automatic algorithm (Ségonne *et al.*, 2004; Reuter *et al.*, 2010). Then, it performs automated Talairach transformation and segmentation of the subcortical white matter and deep gray matter structures (Fischl *et al.*, 2002; Fischl *et al.*, 2004). This step also yields a measure of total intracranial volume. In a second step, the cortical grey matter of each hemisphere is extracted as a single manifold (a folded 'sheet' without holes) to allow the calculation of cortical thickness in a fine grid over the whole cortex. Freesurfer morphometric procedures have been demonstrated to show good test-retest reliability across scanner manufacturers and across field strengths (Han *et al.*, 2006; Reuter *et al.*, 2012) and were also found to accurately align cortical landmarks in children's brains (Ghosh *et al.*, 2010). Because the volumes of the right and left hemisphere were highly correlated, volumes were pooled over hemispheres.

Statistical analyses

For group comparisons, chi-square tests were used for dichotomous variables. Univariate analysis of covariance (ANCOVA) controlling for socio-economic status (SES) if comparing cognitive performance and controlling for intracranial volume if comparing brain volumes was used for continuous variables. Hierarchical multiple regression models with verbal or spatial working memory performance as dependent variables were tested. Each model controlled for sex, age at assessment and SES (first step) as well as intracranial volume (second step). All global and regional brain volumes were added simultaneously in the third step to determine how much variance they accounted for after removing the effect of the control variables. Separate models were used for very preterm and term-born participants.

Participants were assigned to a high or low performing group according to their individual working memory performance relative to the group median. High and low performers were identified separately in the very preterm and the term-born group and for spatial and verbal working memory performance. Two-factorial ANCOVA with birth status and performance group as between-subjects factors (controlling for sex, age at assessment, SES and intracranial volume) was used to investigate differences in global and regional brain volume. The significance level was set at $p \leq .05$ (two-tailed). All analyses were performed with IBM SPSS Statistics 22.0.

Results

Participant characteristics

Demographic and perinatal data are summarized in Table 1. As previously reported for this cohort, all participants had IQ scores in the normal range and normal motor abilities at the time of the assessment (Wehrle *et al.*, 2016).

Table 1. Demographic/perinatal characteristics, working memory performance and global and regional brain volume in children and adolescents born very preterm and at term.

	Very preterm participants (n = 34)	Term-born participants (n = 40)	p	p-value (adjusted)[a)]
Demographic characteristics				
Age, *M* (*SD*), range (in years)	12.8 (1.6), 10.4-16.6	13.1 (2.1), 10.0-16.9	.46	
Sex (male/female)	20/14	20/20	.45	
Socio-economic status[b)], *M* (*SD*), range	2.4 (0.9), 1-4	2.0 (0.9), 1-4	.07	
Perinatal characteristics				
Gestational age, *M* (*SD*), range (in weeks)	29.6 (2.0), 25.1-32.0	≥ 37		
Birthweight, *M* (*SD*), range (in grams)	1300 (340), 840-1990	≥ 2500		
Small for gestational age, *n* (%)	2 (5.9)			
Brain injury[c)]	4 (11.8)	na[d)]		
no brain injuries, *n* (%)	24 (70.6)			
mild brain injuries, *n* (%)	10 (29.4)			
Working memory performance at assessment, *M* *(SD)*				
Spatial working memory (errors)	21.1 (14.5)	17.1 (13.7)	.22	.41
Low performers (n = 17 and 20, respectively)	33.2 (9.4)	27.6 (11.1)	.11	.17
High performers (n = 17 and 20, respectively)	8.9 (5.5)	6.6 (5.1)	.18	.11
Verbal working memory (points)	18.9 (2.4)	20.0 (3.0)	.08	.21
Low performers (n = 15 and 18, respectively)	16.7 (1.7)	17.2 (1.3)	.29	.33
High performers (n = 14 and 19, respectively)	21.2 (.8)	22.7 (1.4)	<.001	< .01
Brain volume at assessment, *M* (*SD*)				
Intracranial volume	1432.1 (167.6)	1463.8 (147.7)	.39	
Ventricles	23.1 (13.5)	17.5 (6.0)	.02	.009
Global cortical white matter	420.5 (44.1)	424.4 (47.7)	.72	.65
Global cortical grey matter	509.5 (52.2)	520.7 (48.5)	.34	.62
Subcortical volume	48.4 (4.0)	49.6 (3.7)	.19	.32
Amygdala	3.3 (.5)	3.3 (.5)	.83	.44
Basal ganglia[e)]	24.0 (2.4)	24.5 (1.8)	.31	.53
Hippocampus	7.4 (.9)	7.7 (.8)	.12	.20
Thalamus	13.7 (1.3)	14.1 (1.3)	.19	.33
Cerebellar white matter	24.4 (3.0)	25.6 (3.0)	.09	.14
Cerebellar grey matter	101.9 (10.9)	102.1 (11.3)	.94	.70
Brain stem	17.3 (1.6)	18.1 (2.0)	.07	.11
Corpus callosum	2.8 (.5)	2.8 (.4)	.91	.99

All brain volumes in cm^3. [a)] p-value (if adjusted for SES (working memory performance) or intracranial volume (brain volume)). [b)] mean of maternal education and paternal education; 1 = highest socioeconomic status. [c)] brain injuries seen on neonatal ultrasound. [d)] parents reported no perinatal complications. [e)] including globus pallidus, putamen, caudate nucleus.

Working memory performance

Overall, no significant difference between the two groups was identified for either spatial or verbal working memory performance (p = .41 and p = .21, respectively, Table 1).

For spatial working memory, group differences for 4-, 6- and 8-box trials were evaluated separately. As reported previously for this cohort (Wehrle *et al.*, 2016), very preterm participants scored comparably to their term-born peers in the trials which placed low and intermediate demands on the abilities (4-box trials: .24 [.6] vs. .38 [.6] errors, p = .28, standardized mean difference adjusted for SES (Lipsey and Wilson, 2001) *(d)* = .22 and 6-box trials: 4.5 [3.8] vs. 4.8 [5.5] errors, p = .56, d = .07). In contrast, clinically relevant spatial working memory deficits ($d \geq .41$ as defined by relevant guidelines (Ferguson, 2009)) were

identified in very preterm participants in the most demanding 8-box trials (16.4 [11.4] vs. 11.9 [9.5] errors, $p = .16$, $d = -.42$).

With regard to verbal working memory, high performing very preterm participants performed significantly poorer than high performing term-born participants ($p < .01$, Table 1).

Relationship between brain volumes and working memory abilities

In the very preterm group, smaller cerebellar white matter ($\beta = -.952$, $p = .003$) and smaller thalamic volume ($\beta = -.632$, $p = .03$) were associated with poorer spatial working memory performance after controlling for sex, age at assessment, SES and intracranial volume. The full model accounted for 38.7% of the variance in spatial working memory performance ($F(14, 19) = 2.486$, $p = .03$, adjusted $R^2 = .387$, Table 2). In term-born participants, the model did not account for a significant amount of variance ($F(14, 25) = 1.629$, $p = .14$, adjusted $R^2 = .184$). Figure 1 depicts the association between spatial working memory performance and the thalamic and cerebellar white matter volume in the two groups.

Verbal working memory abilities were not associated with global and regional brain volume in either group after taking into account sex, age at assessment, SES and intracranial volume (very preterm group: $F(14, 19) = 1.330$, $p = .28$, adjusted $R^2 = .12$; term-born group: $F(14, 25) = 1.672$, $p = .13$, adjusted $R^2 = .19$).

Two-factorial ANCOVA revealed significant interactions between birth status and performance group (spatial working memory) for global cortical white and grey matter volume ($F(1, 66) = 3.922$, $p = .05$ and $F(1, 66) = 6.373$, $p = .01$, respectively). Also, significant interactions were found for thalamic ($F(1, 66) = 5.555$, $p = .02$) and hippocampal ($F(1, 66) = 6.866$, $p = .01$) volume. None of the main effects was significant.

Significant interactions between birth status and performance group (verbal working memory) were found for thalamic ($F(1, 58) = 6.886$, $p = .011$), hippocampal ($F(1, 58) = 6.670$, $p = .01$) and brainstem ($F(1, 58) = 4.445$, $p = .04$) volume. None of the main effects was significant.

Figure 2 illustrates the distinct volume differences between high and low performers in the very preterm and the term-born group.

Table 2. Hierarchical multiple regression predicting errors in spatial working memory task in children and adolescents born very preterm (n = 34).

		B	*SE B*	β	*t*
Step 1					
	Constant	43.362	18.542		2.339*
	Sex	2.322	4.651	.080	.499
	Age at assessment	-3.147	1.450	-.354	-2.171*
	Socio-economic status	6.989	2.671	.430	2.617*
Step 2					
	Constant	47.494	31.345		1.514
	Sex	2.903	5.911	.100	.491
	Age at assessment	-3.138	1.475	-.353*	-2.127*
	Socio-economic status	6.868	2.814	.422*	2.441*
	Intracranial volume	-.003	.018	-.035	-.164
Step 3[a]					
	Constant	-8.710	47.237		-.184
	Sex	-7.215	6.094	-.249	-1.184
	Age at assessment	1.527	2.006	.172	.761
	Socio-economic status	7.606	2.903	.468*	2.620*
	Intracranial volume	0.047	0.024	.547	1.952
	Cortical white matter	-.006	.115	-.019	-.053
	Cortical grey matter	-.031	.088	-.113	-.356
	Cerebellar white matter	-4.646	1.364	-.952**	-3.407**
	Cerebellar grey matter	.637	.407	.480	1.566
	Corpus Callosum	10.418	5.296	.367	1.967
	Brainstem	2.385	2.131	.306	1.119
	Basal ganglia	2.781	1.694	.457	1.642
	Thalamus	-6.871	2.931	-.632*	-2.344*
	Hippocampus	-5.299	2.932	-.338	-1.807
	Amygdala	-2.480	6.386	-.081	-.388

B: unstandardized coefficient; *SE B*: standard error of *B*; β: standardized coefficient; *t*: t-test for individual predictors, *p < .05. **p < .01.

[a] Final model. $F(14, 19) = 2.486$, $p = .03$; $R^2 = .647$ (adjusted $R^2 = .387$).

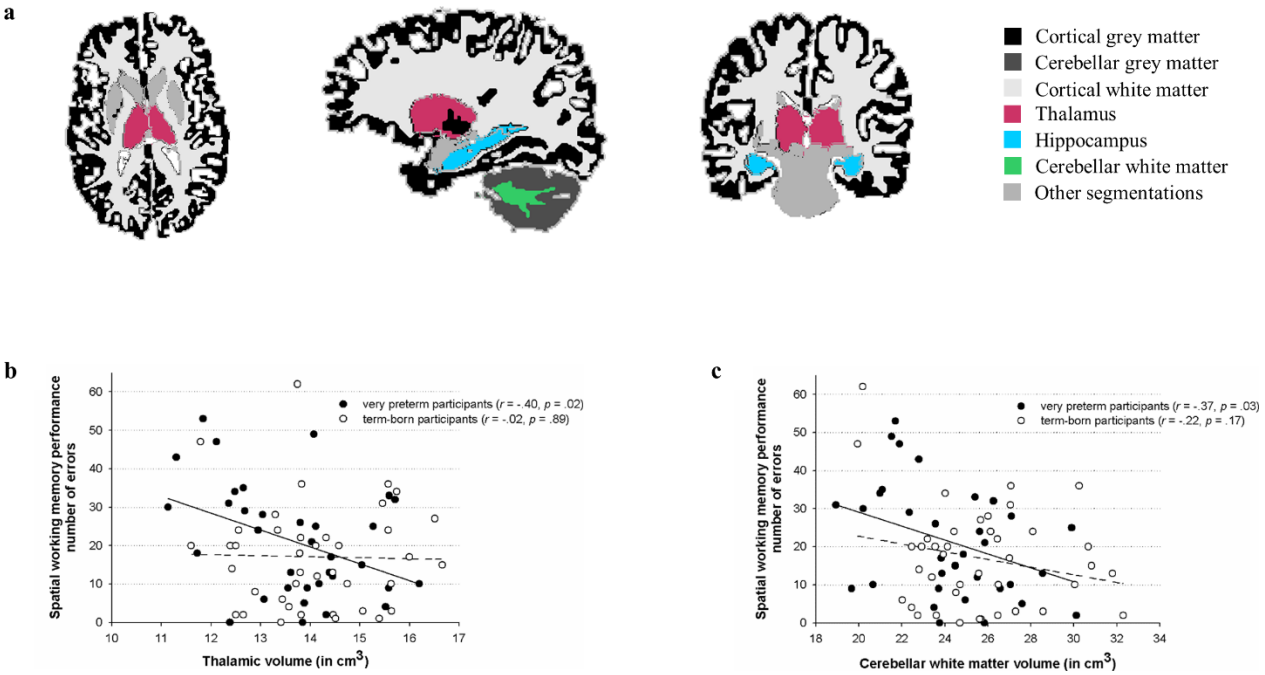

Figure 1. Association between regional brain volume and spatial working memory performance. a) Axial, sagittal and coronal view of the brain of one representative participant. Color-coded are the thalamus, the hippocampus and the cerebellar white matter (using Freesurfer segmentation). Association between spatial working memory abilities and the b) thalamic and the c) cerebellar white matter volume in children and adolescents born very preterm (black dots, solid line) and at term (white dots, dashed line).

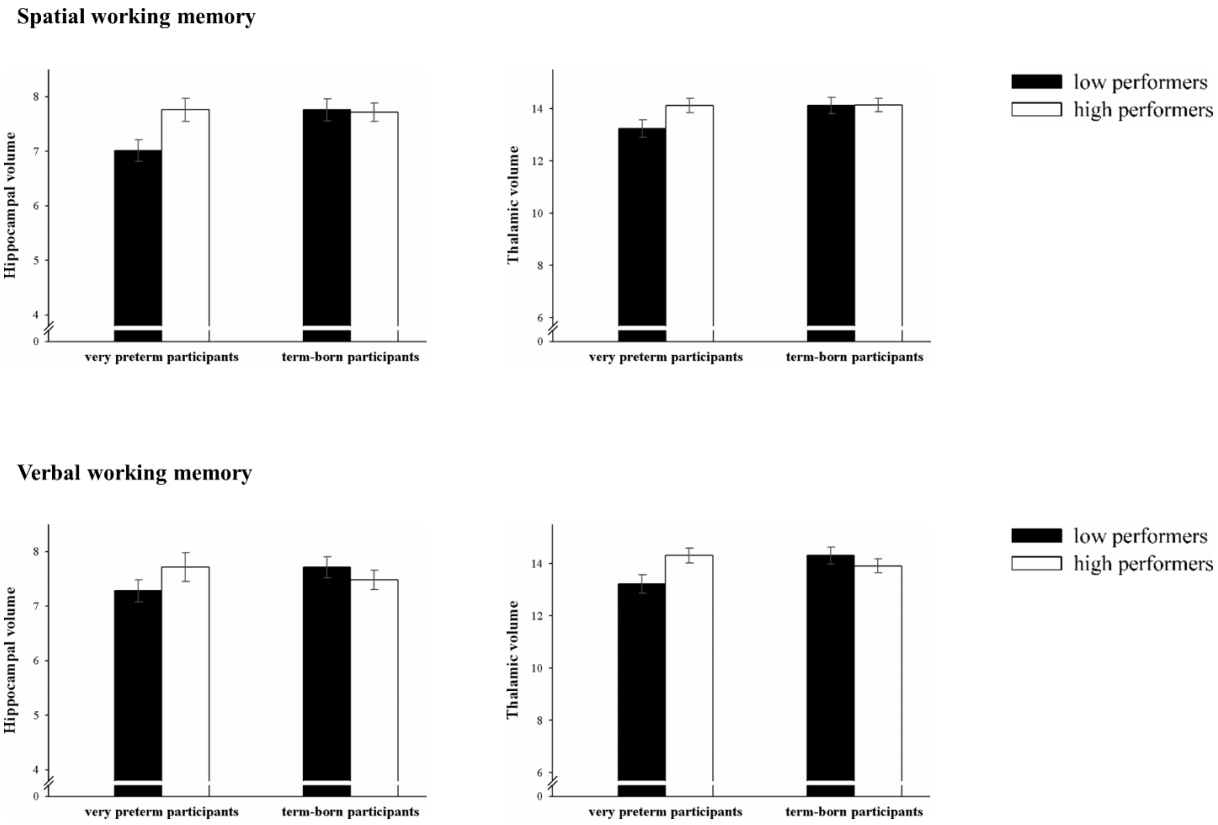

Figure 2. Regional brain volumes (in cm^3) for which two-factorial ANCOVA revealed significant birth status by performance group interactions in spatial and verbal working memory performance. Brain volume is shown separately for low and high performance within the very preterm and the term-born group.

Discussion

This study investigated whether global and regional brain volumes are associated with working memory abilities in a group of children and adolescents born very preterm who exhibit mild working memory deficits despite normal intellectual and motor abilities. Additionally, volumetric differences between high and low working memory performers and potential relations to preterm birth were explored.

Hierarchical regression revealed that brain volume in several subcortical regions was associated with spatial (but not verbal) working memory abilities in children and adolescents born very preterm: Smaller thalamic volume and cerebellar white matter volume predicted poorer spatial working memory performance independent of sex, age at assessment, socio-economic status and total intracranial volume. In addition, volumetric differences between low and high performers were not equal in the very preterm and the term-born group. Notably, low performing very preterm individuals had smaller thalamic and hippocampal volumes compared to both high-performing very preterm and high and low-performing term-born peers. Together, these findings suggest that subcortical volumes, particularly in thalamic, hippocampal and cerebellar regions, may underlie working memory performance and the respective deficits following very preterm birth. Importantly, the subcortical regions identified here have previously been found to be involved in working memory performance in typically developing children (Scherf *et al.*, 2006; Finn *et al.*, 2010; Ullman *et al.*, 2014), despite not being part of the core visuo-spatial working memory network, which consists mainly of cortical fronto-parietal brain regions.

The current study identified the thalamic and cerebellar white matter volume as predictors of spatial working memory abilities in the very preterm but not the term-born group. Previously, in children born very preterm with cystic periventricular leukomalacia (PVL), reduced thalamic volume was found to be associated with working memory impairments (Zubiaurre-Elorza *et al.*, 2012). Also, a functional MRI study reported task-typical patterns of brain activation, mainly in fronto-cingulo-parietal, thalamic and cerebellar areas in young adults born preterm during a working memory task (Daamen *et al.*, 2014). Thus, both the thalamus and the cerebellum have previously been identified as relevant correlates of working memory processes in the very preterm brain. Interestingly, these structures were not associated with working memory performance in term-born peers in the current study. It has been suggested that individual differences may be more readily detectable within groups compared to between groups as pathological processes may be more similar among preterm individuals but rather different from term-born peers (Feldman *et al.*, 2012). Both the cerebellum and the thalamus

are known to be specifically affected by preterm birth (Volpe, 2009a; Ball *et al.*, 2012) and their development is strongly affected in children with diffuse white matter injuries (Boardman *et al.*, 2006), the predominant brain injury after preterm birth affecting up to 50% of all very preterm infants (Volpe, 2003). The potential factors influencing the development of the cerebellum and the thalamus are likely more similar within the very preterm group compared to between the preterm and the term-born group. Consequently, the brain-behavior relationship may only become apparent within the very preterm group. Along the same lines, Zubiaurre-Elorza and colleagues (2012) found strong associations between thalamic volume and working memory performance in very preterm children with cystic PVL while no such association was found in preterm children without cystic PVL. Thalamic development is strongly affected by PVL (Ligam *et al.*, 2009) and the distinct associations seen between the two groups may have resulted from the differential impact on the thalamus in the two groups. The current findings support the notion that the investigation of within group brain-behavior associations may be more sensitive to identify structural correlates of cognitive abilities compared to between group comparisons.

The finding of the smallest hippocampal volume in those very preterm individuals who perform poorly in verbal and spatial working memory tasks is in line with one study in very preterm children which reported smaller hippocampal volume at term-equivalent age to be associated with working memory deficits at early school-age (Beauchamp *et al.*, 2008). In contrast, at early school-age, neither hippocampal volume nor hippocampal development from infancy to early childhood was reported to relate to working memory abilities (Omizzolo *et al.*, 2013; Thompson *et al.*, 2014). A recent review (Nosarti and Froudist-Walsh, 2016) has linked previous findings on structural alterations with findings from functional neuroimaging studies: They concluded that altered hippocampal-cortical networks may underlie working memory deficits after very preterm birth with a shift from the reliance on subcortical, particularly hippocampal regions, to associations with mainly cortical regions across age. The current study is the first to investigate school-aged and adolescent very preterm individuals and the findings suggest that the hippocampus remains a crucial structure related to working memory abilities beyond early childhood, especially if performance level within the group is taken into account. Similarly, one study in young adults born very preterm or with very low birthweight reported an association between hippocampal volume reduction and concurrent working memory problems (Aanes *et al.*, 2015).

While spatial working memory abilities were predicted by several subcortical volumes in the very preterm group, verbal working memory was not associated with either global or

regional brain volume. Importantly however, when taking into account performance level, distinct patterns of volume differences between high and low performers in the very preterm and term-born group were found not only for spatial but also for verbal working memory. Those very preterm individuals with poor verbal working abilities had the smallest hippocampal, thalamic and brain stem volume, thus, suggesting a crucial role of subcortical brain regions for verbal similarly to spatial working memory abilities. This is consistent with a functional MRI study with school-aged children born very preterm with normal general neurodevelopment reporting impaired efficiency in frontal brain areas during a working memory task for younger and low-performing individuals. In turn, no alterations were found in older and high-performing individuals compared to term-born peers (Mürner-Lavanchy *et al.*, 2014). Together, these findings highlight the importance of investigating within group alterations of brain development by taking into account performance level.

The identified working memory deficits in the very preterm group in this study were mild as they were restricted to the spatial modality and only became apparent as task demands increased. Future studies with different working memory tasks, which potentially are more sensitive to detect the mild deficits characteristic of today's very preterm survivors, will have to confirm the importance of the subcortical structures which were identified here.

Limitations

Most brain-behavior associations reported in previous studies in children and adolescents born very preterm have emerged in the context of widespread brain volume reductions in those born very preterm (e.g., Nosarti *et al.*, 2008; Taylor *et al.*, 2011). In contrast, global and regional brain volumes were not significantly reduced in children and adolescents born very preterm in the current study. As early brain injuries disturb subsequent brain development (Volpe, 2009b), the inclusion of only children without any major neonatal brain injuries may partly explain the minimal volume differences between the groups. More importantly, however, the sample size of the current study was limited even if it was comparable to other studies looking at brain-behavior associations after very preterm birth (Griffiths *et al.*, 2013; Mürner-Lavanchy *et al.*, 2014; Aanes *et al.*, 2015). Insufficient power may have constrained the identification of subtle volume differences between the groups, especially as all global and regional brain volumes tended to be smaller in the very preterm compared to the term-born group. Also, the absence of significant group differences in overall verbal working memory may have resulted from insufficient power and future studies should include larger samples to confirm the current findings.

This cohort was assessed cross-sectionally and the investigation of longitudinal brain volume changes, thus, is not possible. In the future, multiple assessments in very preterm individuals across infancy, childhood and into adolescence and young adulthood will improve the understanding of the persistent patterns of altered brain development following very preterm birth and their relation to higher-order cognitive function deficits.

Conclusion

In this study, larger volumes in several subcortical brain regions were associated with better working memory abilities in children and adolescents born very preterm who showed mild working memory deficits despite unimpaired general neurodevelopment. Specifically, the thalamus, the cerebellar white matter and the hippocampus may be relevant structural correlates of the exclusive deficits in higher-order cognitive functions, which frequently affect today's very preterm survivors. A better understanding of the neuronal mechanisms, which underlie these deficits may reveal important targets for neuroprotective agents and may help to identify biomarkers to target those at high risk for developing deficits early with tailored interventions. This may eventually help to close the developmental gap between children and adolescents born very preterm and their term-born peers.

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4.3 Sleep Spindles Predict Schizotypal Personality Traits and Thalamic Glutamine/ Glutamate in Healthy Subjects

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Abstract

Background: Schizophrenia is a severe mental disorder affecting approximately 1% of the worldwide population. Yet, schizophrenia-like experiences (schizotypy) are very common in the healthy population, indicating a continuum between normal mental functioning and the psychosis found in schizophrenic patients. A continuum between schizotypy and schizophrenia would be supported if they share the same neurobiological origin. Two such neurobiological markers of schizophrenia are: 1) a reduction of sleep spindles (12-15 Hz oscillations during non-REM sleep), likely reflecting deficits in thalamo-cortical circuits and 2) increased glutamine and glutamate (Glx) levels in the thalamus. Thus, this study aimed to investigate whether sleep spindles and Glx levels are related to schizotypal personality traits in healthy subjects.

Methods: Twenty young male subjects underwent two all-night sleep EEG recordings (128 electrodes). Sleep spindles were detected automatically. After those two nights, thalamic Glx levels were measured by magnetic resonance spectroscopy. Subjects completed a magical ideation scale to assess schizotypy.

Results: Sleep spindle density was negatively correlated with magical ideation ($r = -0.64$, $p<0.01$) and thalamic Glx levels ($r = -0.70$, $p<0.005$). No correlation was found between Glx levels in the thalamus and magical ideation ($r = 0.12$, $p>0.1$).

Conclusions: The common relationship of sleep spindle density with schizotypy and thalamic Glx levels indicates a neurobiological overlap between non-clinical schizotypy and schizophrenia. Thus, sleep spindle density and magical ideation may reflect the anatomical and efficiency of the thalamo-cortical system that shows pronounced impairment in patients with schizophrenia.

Introduction

Schizophrenia is a multifaceted neuropsychiatric disorder that affects approximately 1% of the worldwide population (National Institute of Mental Health, 2013). However, a growing body of evidence suggests that there is a considerably higher incidence of psychosis-like signs (e.g. delusion-like beliefs) in non-clinical populations, a characteristic commonly referred to as schizotypy or psychosis proneness (van Os *et al.*, 2009).

A psychosis continuum entails that symptoms observable in psychotic disorders (e.g. schizophrenia) can be obtained in non-clinical populations and that the presence of psychosis-like symptoms are not necessarily bound to the presence of the disorder (van Os *et al.*, 2009). This theory is supported by studies reporting that schizotypal personality traits and schizophrenia may share common genetic and neurobehavioral features (Vollema *et al.*, 2002; Noguchi *et al.*, 2008; Corlett and Fletcher, 2012). Thus, several studies provide evidence for a genetic continuity between schizophrenia and non-clinical schizotypy (Kendler *et al.*, 1995; Vollema *et al.*, 2002). For example, Vollema et al. (2002) demonstrated that biological-genetic vulnerability to schizophrenia was associated with the positive dimension of a schizotypy questionnaire. Other studies showed that relatives of schizophrenia patients score higher on schizotypy scales than subjects without familial risk (Kendler *et al.*, 1995). In addition, an overlap between schizotypy and schizophrenia was reported in neurocognitive measures that are deficient in patients with schizophrenia. For instance right hemispatial inattention, a neurocognitive deficit frequently observed in schizophrenia, was associated with schizophrenia-like experience and beliefs in healthy young male students (Brugger and Graves, 1997; Kalaycioglu *et al.*, 2000; Taylor *et al.*, 2002). In these studies, the magical ideation scale was used to assess quantitatively a person's proneness to schizophrenia-like experience and thoughts (positive dimension of schizotypy; Eckblad and Chapman, 1983). Therefore, the continuum of magical ideation is psychometrically relevant even for completely healthy subjects. Nevertheless, for schizotypy to represent an attenuated form of psychosis it would need to have the same neurobiological basis. Apart from testing the continuum model, exploring the neurobiological origin of psychosis-like signs in healthy subjects may further contribute to the elucidation of the neurobiological mechanism and anatomical structures underlying psychosis. In addition, investigations in sub-clinical groups may foster the search for the most sensitive neurophysiological and behavioral spectrum markers for schizophrenia. Finally, it is often difficult to distinguish whether an aberrant neurobiological marker in schizophrenic patients is a primary cause of the disease or a result of medication or disease history. Thus, studies in healthy subjects may help to overcome this difficulty.

Based on several findings, schizophrenia has been conceptualized as a network disorder mediated by abnormal brain connectivity and disrupted neuronal communication (Anticevic *et al.*, 2014; Samartzis *et al.*, 2014). In particular, thalamo-cortical deficits have been reported in schizophrenia (Andreasen *et al.*, 1994; Stephan *et al.*, 2006; Anticevic *et al.*, 2014). The sleep spindle, a thalamo-cortically generated phasic oscillation between 12-15 Hz during non-rapid eye movement sleep (NREM), putatively reflects anatomical and functional differences of the thalamo-cortical system (De Gennaro and Ferrara, 2003; Lustenberger *et al.*, 2012). Patients with schizophrenia show a remarkable decrease in sleep spindles, likely reflecting deficits in thalamo-cortical circuits (Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010; Wamsley *et al.*, 2012). Therefore, sleep spindles are thought to be an electrophysiological marker for the integrity of the thalamo-cortical system. Moreover, Ferrarelli et al. (2007) reported a 90% separation of schizophrenic patents and healthy subjects based on spindle measures. Collectively, sleep spindles seem to be a promising neurobiological spectrum marker of schizophrenia.

Increased glutamine (Gln) and glutamate levels (Glu; sum of both denoted as Glx) in the thalamus and the anterior cingulate have also been found in medicated and non-medicated patients with schizophrenia and subjects at high risk for schizophrenia (Theberge *et al.*, 2002; Theberge *et al.*, 2003; Tandon *et al.*, 2013). Thus, altered brain glutamatergic transmission (e.g. disinhibition of glutamate release in the cortex) is proposed as a primary neurochemical marker for schizophrenia (Stone *et al.*, 2007). Pharmacological models of schizophrenia can be obtained by non-competitive NMDA receptor glutamatergic antagonists (e.g. ketamine), which affect behaviour and induce schizophrenia-like manifestations in animal models and humans (Bubenikova-Valesova *et al.*, 2008). These models provide evidence that abnormal glutamatergic transmission might be caused by NMDA receptor blockage in GABAergic interneurons in the thalamus that leads to a disinhibition of glutamate release in the cortex (Moghaddam *et al.*, 1997; Lorrain *et al.*, 2003). Since these thalamo-cortical circuits are also involved in the spindle generation and synchronization, glutamate levels in the thalamus may be related to sleep spindles and schizotypal personality traits.

Assuming that delusion-like beliefs and schizophrenia-like experiences in healthy subjects may have the same neurobiological origin as in patients with schizophrenia, our study aimed to investigate whether sleep spindles are associated with thalamic glutamate and glutamine (Glx) levels in healthy volunteers, and whether Glx levels and sleep spindles are related to magical ideation.

Methods

Participants

Twenty healthy male subjects (age: 23.3 ± 2.1 years; mean ± SD) were recruited by advertisement at the University of Zurich and ETH Zurich. Only male subjects were included since in female subjects spindle activity varies systematically across the menstrual cycle (Driver *et al.*, 1996). Participants with family history of psychopathology, chronic diseases, current use of psychoactive agents or other medications were excluded (telephone and questionnaire screening). Furthermore, we applied the schizotypal personality questionnaire (Raine, 1991) in German (Klein *et al.*, 1997) to exclude subjects at high risk for schizotypal personality disorder. The scores of all subjects were in the normal or even lower range (standardized values: -1.1 ± 0.64 SD. Norm values provided by Klein et al. (1997), n = 649 male healthy young adults). Thus, none of our subjects was at a higher risk for schizotypal personality disorder. They were further non-smokers and right handed. All subjects were normal, healthy sleepers as verified with a screening night (sleep efficiency > 80%). To ensure stable conditions, subjects were required to maintain a regular sleep-wake schedule (8 h time in bed, according to scheduled bedtime in the lab) and to abstain from caffeine, naps and alcohol three days before the study nights. Compliance was controlled by breath alcohol test, wrist-worn actometers and sleep logs. The procedures were approved by the cantonal ethic commission in Zurich (Switzerland), and the study was performed according to the Declaration of Helsinki. All participants gave written informed consent to participate in the experiment.

Procedure

Each participant underwent two study nights two weeks apart with bedtimes either at 22:50-06:50 or 23:40-07:40. All-night high-density EEG was recorded during both nights. Subjects underwent a magnetic resonance spectroscopy measurement 11.0 ± 8.2 days after the second study night and completed a magical ideation scale.

Polysomnography

High density EEG (128 electrodes; e.g., Lustenberger and Huber, 2012), electrooculogram (EOG) and submental electromyogram (EMG) were continuously recorded during the 8-h night-time sleep episode. The signals were digitized at 500 Hz (filters: 0.01-200 Hz), referenced to Cz. For further analysis the EEG was band-pass filtered (0.5-40 Hz) and re-sampled at 128 Hz.

The sleep stages were scored for 20-s epochs according to standard criteria (Iber *et al.*, 2007) and artefacts were identified on a 20-s basis by visual inspection and a semiautomatic procedure. The data was re-referenced to the average reference of all good quality EEG channels above the ears (109; of these, on average, 7 channels per subject were of insufficient quality). Thereafter, for topographical analysis, spindle values (see section spindle detection) of bad channels were interpolated using a spherical interpolation provided by EEGLAB toolbox (Delorme and Makeig, 2004).

Spindle detection

Sleep spindle detection was performed according to the detection algorithm of Ferrarelli et al. (2007). We thereby focussed on the first hour of artefact-free NREM sleep. We selected this time interval because it includes the same number of epochs for all subjects and belongs to the most consolidated part of sleep. In short, the EEG signal was band-pass filtered between 12-15 Hz. A sleep spindle was detected in the rectified signal if the signal amplitude exceeded an upper threshold that was defined relative to the mean signal amplitude. An upper threshold of 5 times the mean signal was determined to result in the best spindle detection after visual inspection of spindle density values that were comparable with previous studies (Dijk *et al.*, 1993; Nicolas *et al.*, 2001). Beginning and end of sleep spindles were set when the signal around the peak amplitude dropped below a lower threshold (2 times the mean signal). We focussed our analysis on sleep spindle density (number per min NREM sleep), because this measure is one of the most affected in patients with schizophrenia (Ferrarelli *et al.*, 2010; Wamsley *et al.*, 2012). An illustration of an EEG trace with detected sleep spindles is shown in Supplemental Figure 1 and averaged spindle density number in Supplemental Figure 2. Using this algorithm with the specific thresholds the number of detected sleep spindles per time (density) was comparable with several other studies (Gaillard and Blois, 1981; Dijk *et al.*, 1993; De Gennaro *et al.*, 2000; Nicolas *et al.*, 2001) (density at C4 [average referenced EEG]: 3.8921 ± 0.265 spindles per min). Importantly, so far no "gold standard" for spindle detection is available. This is also supported by the fact that reported spindle density values and the chosen detection algorithms vary across studies.

MR Spectroscopy

MR spectroscopy studies were performed with a 3 T GE HDxt MRI scanner (GE Medical Systems, Milwaukee, WI, USA) equipped with TwinSpeed gradients. Single voxel ^{1}H MR spectra were acquired from a 20x20x20 mm^3 voxel of interest (VOI) positioned in the left thalamus as done in previous studies (Theberge et al., 2002; Theberge et al., 2003; Stone et al., 2009; Fusar-Poli et al., 2011) using a Point RESolved Spectroscopy (PRESS) sequence with an echo time of 35 ms and a repetition time of 3 s. The left thalamus was chosen to avoid chemical shift displacement artefacts, which may result in unwanted excitation of the water in ventricular CSF, outside the prescribed voxel. Sixty-four spectral averages were acquired for each spectrum using an 8-channel receive-only head coil, resulting in an acquisition time of four minutes (illustrative spectra and the selected voxel of the thalamus can be found in Supplemental Figure 3). The scan protocol also included structural imaging (T1, T2 and DTI). T1-weighted images were acquired with a 3D fast inversion-recovery prepared spoiled gradient acquisition in the steady state (IR-SPGR), with inversion time (TI) = 600 ms, echo time (TE) = 4.25 ms, repetition time (TR) = 11.4 ms, flip angle = 8 degrees, and an isotropic voxel resolution of 1 mm, used for localization of the spectroscopy voxels. The volumetric IR-SPGR images were also segmented into grey matter, white matter and CSF maps using statistical parametric mapping (SPM8, Wellcome Dept of Cognitive Neurology) to correct the spectroscopy results for partial volume CSF contamination.

Total scan time was 25 minutes. Subjects were told to stay awake during the whole scan procedure. We spoke to the subjects between each scan to verify that they were still awake. They all confirmed that they did not fall asleep during these MRS scans or before.

Water scaled metabolite concentrations were derived with the LC Model version 6.2-4 (Provencher, 1993). Each MR-spectrum was visually inspected for the presence of artefacts or fitting errors. Poorly fitted metabolite peaks (Cramer-Rao minimum variance bounds of more than 10% for Cre or N-acetyl aspartate [NAA] or more than 20% for any of the other metabolites) and spectra with visible artefacts were excluded from further analysis. For the included spectra the average signal to noise ratio (SNR) was 19.2 (± 3.4 SD) and the linewidth 5.9 Hz (± 0.8 SD). Due to the tight spectral overlap between glutamate Glu and Gln at 3 T (Tkac et al., 2001; Stone et al., 2009), the sum of both, Glx, was used to investigate the link between glutamate levels, spindle rhythms and schizotypy. The concentrations were corrected for partial volume contamination of CSF (Kreis et al., 1993), using the correction factor given in Chowdhury et al. (2015). To control for differences in the fractions of grey (GM) and white

matter (WM) within the voxel we covaried in an additional analysis for the GM/WM fraction (Edden et al., 2012; Chowdhury et al., 2015) in a partial correlation design.

Schizotypal properties

We used an adapted version of the magical ideation scale developed by Eckblad and Chapman (Eckblad and Chapman, 1983) which is a validated questionnaire commonly used to asses proneness to schizophrenia-like experience and thoughts (indicator for schizotypy or schizophrenia-proneness). This questionnaire originally consisted of 30 true/false questions, of which we selected 10 specific questions (items 4, 11, 15, 19, 20, 24, 25, 27, 28, and 30; English version of the scale printed in full (Eckblad and Chapman, 1983)). This selection was based on an item-total correlation analysis ($n > 1000$) of the original questionnaire (Eckblad and Chapman, 1983) whereby all single items were correlated to the sum of the others. We selected the 10 items with the highest correlation coefficients, because high item-total correlation values contribute more to a scale's reliability and may be considered more representative of the concept "magical ideation" than low item-total correlation values (unpublished data provided by Dr. Peter Brugger). Instead of true/false answers subjects had a 6-point rating scale for each question ranging from "strongly disagree" (0 points) to "strongly agree" (5 points). This rating scale allows a more fine-tuned assessment of magical thinking. Scores of the 10 questions were added and a higher total score (maximal 50 points) represents a more distinct occurrence of schizophrenia-like experiences and beliefs.

Of note, this adapted version of the Eckblad and Chapman questionnaire (Eckblad and Chapman, 1983) was not validated so far. However, the 10 selected questions best represent the concept of "magical ideation". But, the more fine-tuned scale is difficult to compare with the "yes/no" questions of the original Eckblad and Chapman scale (Eckblad and Chapman, 1983). To approximate this "yes/no" type of answers, we additionally rated a question as a "yes" if the subject scored ≥ 3 and as a "no" if the subject scored < 3.

Statistics

All spindle values were averaged for both study nights to obtain more stable spindle measures. Pearson correlations were used to assess relationships between sleep spindle measures, schizotypal properties and Glx levels in the left thalamus. Topographical distributions of *r*-values are shown for correlations including the spindle measures. For topographical analysis we applied statistical nonparametric mapping (SnPM) using a supra-threshold cluster analysis to control for multiple comparisons and to define specific regions of

interest (Nichols and Holmes, 2002; Ferrarelli *et al.*, 2010). Thus, the neighbouring electrodes that were above/below a significant r-value of 0.54/-0.54 (corresponding to a p-value of 0.025 for n=17, Bonferroni controlled p-value for 2 correlation topographies) and exceeded the 90th percentile cluster size given by the permutation analysis were considered significant and included in further analysis (regions of interests). Correlations of these specific regions of interest (illustrated in the scatter plots) were considered significant with p-values<0.05. For the sleep spindle measures one subject had to be excluded due to bad sleep and data quality. Two subjects were excluded from MRS analysis due to poor MR-spectrum quality. One subject did not complete the questionnaire and one subject was a correlation outlier with a questionnaire score that was more than 2.5 times the standard deviation above the mean (30 points). The number of subjects included in the correlation analysis is provided in the respective figures.

Results

In the magical ideation questionnaire, our subjects (n=20) reached on average 10 points (± 7 SD, out of 50 points) and answered 1.74 questions (± 1.79 SD) with "yes" (range of questions scored with"yes" is 0-6 out of 10 questions). Chmielewski et al. (Chmielewski *et al.*, 1995) provided norm data for the Eckblad and Chapman scale (Eckblad and Chapman, 1983) for white male students (n=3112). Their subject answered on average 30.3% of the questions with "yes", in our sample only 17.9% of the questions were answered with "yes". As the number of questions differs between the original and the adapted assessment tool, (30 vs. 10 questions) this value is difficult to compare, but seems to be lower than the norm. According to Eckblad and Chapman (Eckblad and Chapman, 1983), subjects that scored 2SD above the mean (4.4% of all participants) were rated to be at higher risk for psychosis. In our study, only one subject was clearly deviant from the mean (60% of the questions answered with "yes", 30 of 50 possible points on the fine-tuned scale) and a correlation outlier, and was therefore not included in the correlation analysis.

We first explored the relationship between sleep spindle density in the frequency range of 12 to 15 Hz with magical ideation and thalamic Glx levels (Figure 1).

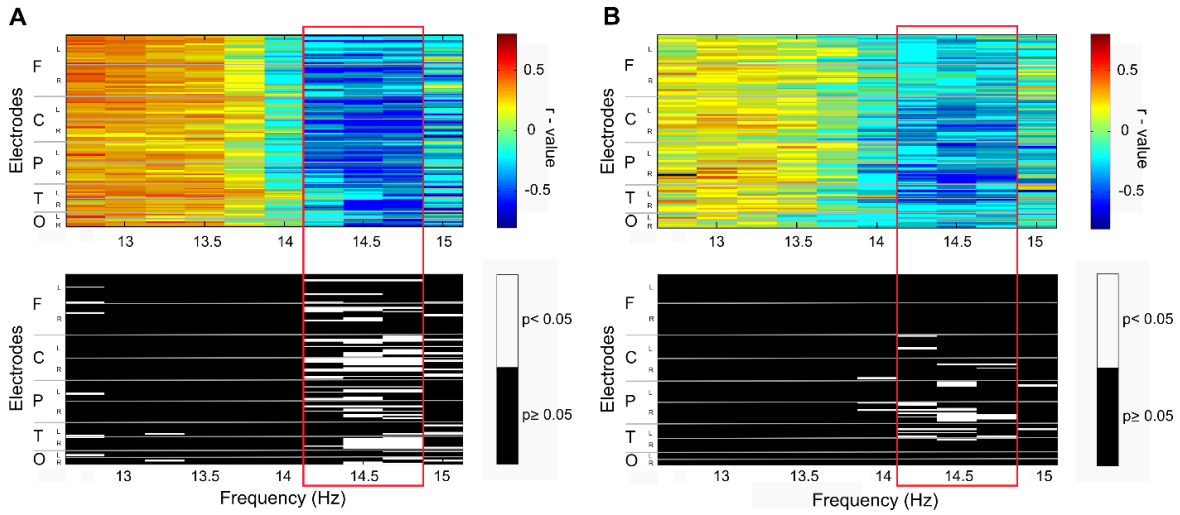

Figure 1. Fast spindle density is inversely correlated with magical ideation and thalamic Glx levels. Heat plots of the correlation coefficients and corresponding statistics for the relationship between spindle density of the first hour of NREM sleep and A) magical ideation score (n = 17) B) thalamic Glx levels corrected for CSF (n = 17). The x- axis represents the frequency bins where sleep spindles were detected by our algorithm (supplementary Figure 1 and 2). The y-axis indicates the 109 high density EEG electrodes grouped into sets of electrodes that were close in distance to the 10-20 system configuration. F: Frontal, C: Central, P: Parietal, T: Temporal and O: Occipital, L: Left, R: Right. The red rectangle highlights the frequency bins that showed significant (white bars, $p < 0.05$) correlations over pronounced clusters of electrodes.

To control for multiple comparisons, only neighbouring frequency bins that demonstrated pronounced regional clustering of significant correlations with both magical ideation and Glx levels in the thalamus were considered for further analysis (red rectangles in Figure 1, q.v. Supplemental Figure 1 for spindle density distribution). Visual inspection revealed pronounced clusters of correlations between magical ideation/thalamic Glx and sleep spindle density in the 14.25-14.75 Hz range (3 0.25-Hz bins; Figure 1). Based on these results, we further investigated the topographical distribution of the relationship (correlation) between spindle density in the frequency range of 14.25-14.75 Hz and magical ideation, and thalamic Glx levels, respectively. Magical ideation, a marker for a person's proneness to schizophrenia-like experience and thoughts, was negatively correlated with spindle density in a widespread cluster of centro-parietal electrodes (Figure 2 A), with the highest R^2-value reaching 0.46. These correlations even persisted when using the adapted "yes/no" score (see methods, data not shown).

In addition, higher thalamic Glx were associated with lower spindle density levels for a right parieto-temporal cluster (Figure 2 B). In this cluster, the highest R^2-value was 0.59. While approximately half of the voxel contains thalamic structure, it is clear that other subcortical areas are also included within the voxel (q.v. Supplemental Figure 3). Thus, in an additional analysis we also included the GM/WM ratio (0.49 ± 0.12) as a covariate in our correlation to test whether differences in tissue fraction may account for the observed relationship between Glx and sleep spindles (Supplemental Figure 4). The results were comparable to those obtained without applying the GM/WM fraction as a covariate (highest R^2-value was 0.58 for partial correlation).

Finally, we investigated the relation between Glx level in the thalamus and magical ideation and observed no significant correlation between these two measures (Figure 3).

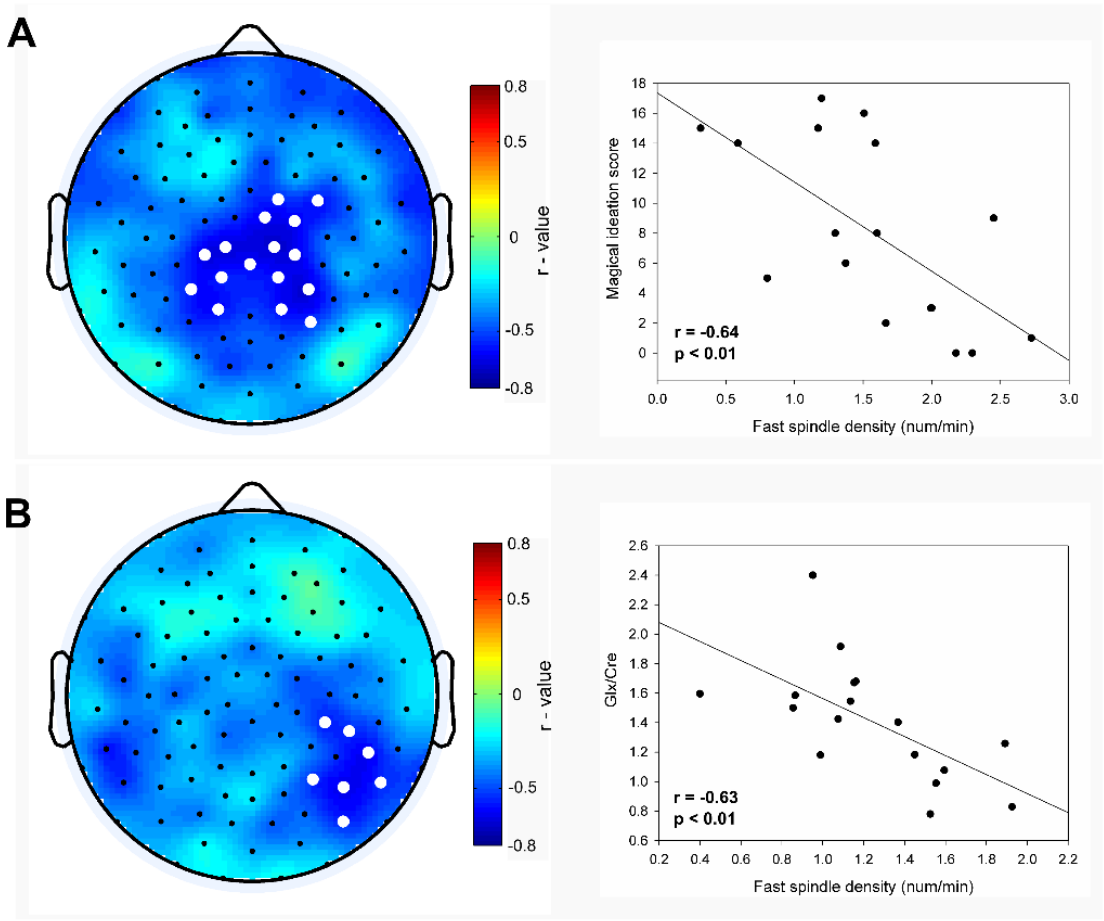

Figure 2. Illustration of the correlation between fast spindle density (14.25-14.75 Hz) and **A**) magical ideation score (n=17), and **B**) thalamic Glx levels corrected for CSF (n=17). Topographical distribution of r-values is plotted on the planar projection of the hemispheric scalp model with negative correlations reflected in blue. White dots indicate significant electrode clusters after controlling for multiple comparisons. The scatter plots further demonstrates the significant correlation for the highlighted electrode clusters.

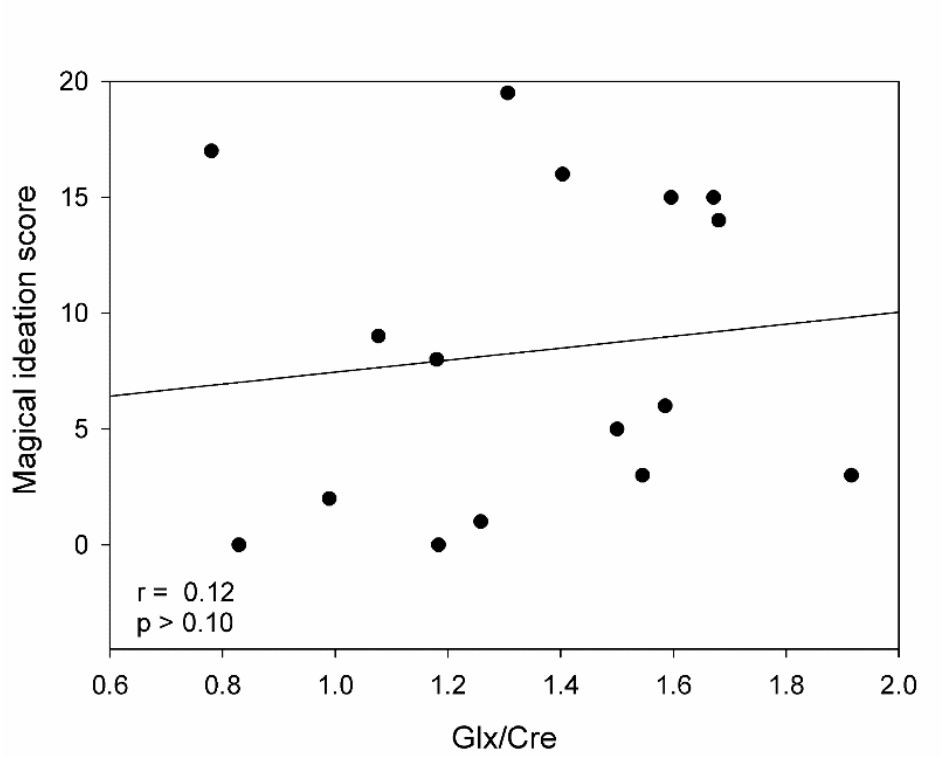

Figure 3. Correlation between thalamic Glx levels corrected for CSF and the magical ideation score was not significant (n = 16).

Discussion

In this study we combined for the first time high density sleep EEG and MRS measures of Glx to investigate neurobiological correlates of schizotypy. We found that fast sleep spindle density was inversely related with magical ideation and thalamic Glx.

Reduced sleep spindling (e.g. density and power) has been reported in schizophrenia (Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010; Wamsley *et al.*, 2012). Thus, the negative relationship between magical ideation, which reflects the proneness to delusion like beliefs, and sleep spindle density is consistent with previous findings in patients with schizophrenia. Importantly, we found this relationship in healthy young subjects that do not suffer from schizophrenia or schizotypal personality disorder, implying that schizophrenia-like perceptual experiences in healthy and psychotic symptoms in patients with schizophrenia seem to overlap at the neurobiological level. This finding supports the continuum model suggesting that unusual but non-clinical beliefs may represent a milder form of the clinical delusions found in severe mental illness (van Os *et al.*, 2009). This line of research provides evidence that schizotypal personality traits and schizophrenia may share common genetic, neurophysiological, neurocognitive and neurobiological features (Vollema *et al.*, 2002; Noguchi *et al.*, 2008;

Aichert *et al.*, 2012; Corlett and Fletcher, 2012). To date only very few studies investigated the neurobiological overlap between schizophrenia and schizotypy (Aichert *et al.*, 2012; Corlett and Fletcher, 2012). Corlett and Fletcher (Corlett and Fletcher, 2012) were able to relate schizotypy to neuronal responses in an associative learning task. They reported in healthy subjects a negative correlation between magical ideation and fronto-striatal prediction error signal, which describes the mismatch between what we expect and what we experience in a given situation, measured with functional magnetic resonance imaging (fMRI). Importantly, a disrupted dorsolateral prefrontal prediction error signal was also found in patients suffering from psychotic illness (Corlett *et al.*, 2007). The investigation of neurobiological overlap between schizophrenia and non-clinical schizotypy is important to disentangle neural mechanism and underlying structures that are involved in the generation of psychosis. Both sleep spindles and magical ideation may depend on the anatomy and efficiency of the thalamo-cortical system, e.g. the number, strength and myelinisation of thalamo-cortical fibers (Miller, 1994; Fogel *et al.*, 2007a). The reticular nucleus of the thalamus is the generator of sleep spindles (Kandel and Buzsaki, 1997; Steriade, 2006) and plays a significant role in sensory input gating, processing and filtering of information (McAlonan and Brown, 2002). Schizophrenics are thought to be overwhelmed with information and stimuli due to deficient thalamo-reticular circuits that may lead to delusion and hallucination (Andreasen *et al.*, 1994; Crail-Melendez *et al.*, 2013). Thus, anatomical and functional variations of the thalamus are likely reflected in differences in sensory gating and filtering of information and stimuli that may or may not lead to schizophrenia-like perceptual experiences. Collectively, fast sleep spindle density seems to be a promising and sensitive spectrum marker for schizophrenia. Moreover, our findings indicate that sleep spindle deficits and therefore thalamo-cortical aberrations seem to be a primary marker for schizophrenia, because the relationship in healthy subjects is not confounded by medication or history of disease.

Our data further revealed that fast spindle density was negatively correlated with thalamic Glx. This relationship in healthy subjects is in line with the observation that patients with schizophrenia and subjects at high risk for this illness show increased thalamic Glx. Of note, when comparing the results of the present study to those of previous studies it is important to take into account the different thalamic voxel sizes employed. The voxel used in the present study also included non-thalamic subcortical structures (q.v. Supplemental Figure 3). However, even when we controlled for potential differences in voxel composition (covaried for GM/WM fraction (Edden *et al.*, 2012; Chowdhury *et al.*, 2015)) in an additional partial correlation

analysis the results were comparable to those obtained without applying the GM/WM fraction as a covariate.

Most thalamic afferents and efferents in the thalamus are glutamatergic and have a functional role in modulating thalamic activity during wakefulness and sleep (Steriade *et al.*, 1997). In combination with other neurotransmitters, glutamate can facilitate the transition of the thalamic system from sleep to wakefulness and consequently from a rhythmic burst mode to a tonic single spike mode (Steriade *et al*., 1997). The thalamo-cortical system is responsible for sleep spindle generation whereby thalamo-reticular neurons that fire in the burst-spike mode trigger sleep spindles (Bazhenov *et al*., 2000). Thus, increased thalamic Glx levels during sleep may reflect more activating inputs to the thalamus (brainstem and/or cortical) that suppresses rhythmic burst firing of the thalamus and therefore reduce the generation of sleep spindles. In the present study, the participants were awake during the MRS measurements so we do not know the Glx levels during sleep, and we are further unable to distinguish the origin of the apparent increase in Glx levels in participants scoring high in magical ideation. Another interesting explanation could be that subjects with high thalamic Glx levels (in the awake state) were more aroused in wakefulness and that this tendency toward hyperarousal carried over to the sleep state. However, we were not able to relate the thalamic Glx levels to signs of arousal during sleep (e.g. wake after sleep onset or sleep depth [SWA]).

Increased thalamic Glx may result from several different processes (e.g. increased glutamatergic release or less reuptake of Glu) and Glx levels reflect the changes of both Glu and Gln (Helms *et al.*, 2006). There is evidence that 80-100% of the Glu that is released as a neurotransmitter at the synapse is directly cycled to Gln in the astrocytes, such that Gln levels might reflect the degree of Glu release at a specific region, e.g. thalamus (Theberge *et al.*, 2002; Rothman *et al.*, 2003). However, in addition to Glu playing an important role in neurotransmission it also is involved in metabolic processes. Moreover, the MRS visible Glx signal includes both pools of Glu as well as Gln. Therefore, the Glx concentration measured with MRS may not provide a direct measurement of excitatory neurotransmission.

Interestingly, MRS studies performed at 4T, which provides clear separation of Gln and Glu peaks, mainly found increased thalamic Gln in schizophrenic patients, which might point to an increased Glu release in the thalamus (Theberge *et al.*, 2002; Rothman *et al.*, 2003; Theberge *et al.*, 2003). Moreover the MRS visible Glx signal includes both intra- and extracellular pools of Glu as well as Gln. However, due to the overlap of Glu and Gln at 3T we are unable to separate Glu and Gln levels in our study. While well-fitted Glu values were derived from the MR spectra in all subjects, Gln was not well-fitted in many subjects,

suggesting that in these cases the apparent Glu values may be contaminated with Gln. For this reason, Glx was selected as a more consistent measurement of glutamate across all participants. Admittedly, the hypothesized biological mechanism explaining the relationship between thalamic Glx and the sleep spindles remains speculative and further studies are needed to disentangle the underlying mechanisms. Nevertheless, compelling evidence exists that deficient glutamatergic neurotransmission and spindle generation are found in schizophrenic patients (Moghaddam *et al.*, 1997; Theberge *et al.*, 2002; Lorrain *et al.*, 2003; Theberge *et al.*, 2003; Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010; Tandon *et al.*, 2013). As mentioned in the introduction, abnormal glutamatergic transmission might be caused by NMDA receptor blockage on GABAergic interneurons in the thalamus (e.g., Moghaddam *et al.*, 1997; Lorrain *et al.*, 2003). Thus, Glx levels and sleep spindle density might be tightly coupled through the thalamic system.

While a negative relationship between sleep spindle density and magical ideation/Glx levels was evident over the whole cortex, this correlation only reached significance in central or parieto-temporal electrodes and the correlation was further restricted to the high spindle frequency range (14.25-14.75 Hz). Ferrarelli et al. (2010) demonstrated that the effect size in spindle measure differences between patients and schizophrenia was most pronounced for spindle number (is related to density) and integrated spindle activity (comprising a combination of spindle parameters). When focussing on the first hour of NREM sleep, Ferrarelli et al. (2007) described a spindle reduction (e.g. power, number) in schizophrenics that was, at least for the power, also restricted to the high frequency range (13.75-15.00 Hz) and to a central cluster. Cortical topography of sleep spindles demonstrate that the majority of sleep spindles over centro-parietal regions are around 14 Hz whereas slow spindles around 12 Hz (De Gennaro and Ferrara, 2003) are most pronounced over frontal regions, thus possibly explaining why the correlation in our specific regions was restricted to the high spindle frequency range. Interpretation of these regions should be done cautiously, since we cannot directly deduce underlying source of spindle activity from cortical EEG topography, especially since subcortical regions like the thalamus are involved in its generation. In addition, visually inspecting the topographical distribution of correlation coefficients indicates that the effect is rather global. Thus, we belief the emerging cluster in the right hemisphere is to some extent dependent on statistical power and the possible meaning of the region that reaches significance should not be overestimated.

Finally, thalamic Glx did not predict magical ideation. Therefore, sleep spindle density seems to be a more sensitive marker for schizotypal personality traits, at least in healthy subjects. Tandon et al. (2013) reported a significant positive correlation between Glx levels in the thalamus with measure of schizotypy in subject at high familial risk for schizophrenia, but none in healthy control subjects. It is possible that subjects showing extreme/high values of schizotypy and thalamic Glx levels would show a significant correlation.

Our findings need to be considered in the context of several methodological limitations. Some adaptations and differences of the methods (EEG, MRS and magical ideation scale) compared to other studies were implemented for an optimization to our data and experimental design. Especially for the MRS measurements, different factors might have influenced our data. More specifically, even though carefully controlling for CSF and partial grey to white matter ratio, some of the voxels selected might have been derived from different brain regions than the thalamus.

Furthermore, our results should be considered in the context of a relatively small number of subjects that may not be representative of typical control values. Thus, a larger study would help define the normal variation.

Finally, whether our findings might apply to high risk subjects is unclear, since our healthy subjects scored below the norm of magical ideation. Thus, future studies should investigate these relationships in subjects with schizotypal personality disorder or relatives of schizophrenic patients.

In conclusion, our results support the notion that schizophrenia-like experiences possibly exists as a part of a continuum and depend on the same neurobiological system as psychotic experiences in schizophrenia. Thus, sleep spindle density and magical ideation may reflect the anatomy and efficiency of the thalamo-cortical system that shows pronounced impairment in patients with schizophrenia. In the future, sleep spindle measures might be used as objective markers for schizophrenia. In addition, non-clinical groups seem to be ideally suited to elucidate the neurobiological origin of schizophrenia-like behaviour not confounded by therapy and disease history.

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Supplemental Figures

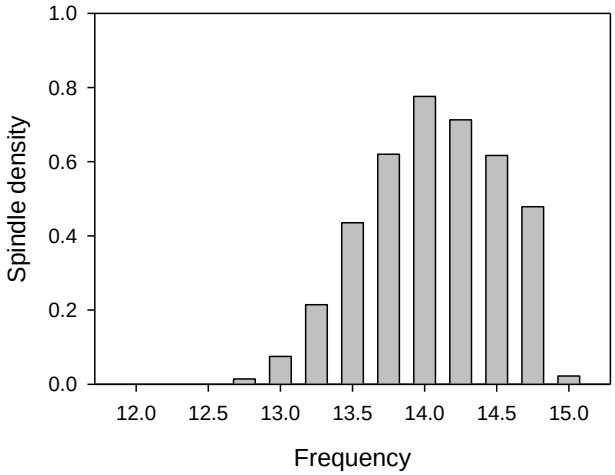

Figure 1. Histogram of spindle densities for 0.25 Hz frequency bins between 12-15Hz. Spindle densities (number/min) are averaged for all 109 electrodes and subjects. Please note that between 12-12.5 Hz no spindles were detected and that at 15Hz a clear drop is visible. This limited detection is likely a cause of the filter settings (12-15Hz) that prevented accurate detection at the frequency boarders.

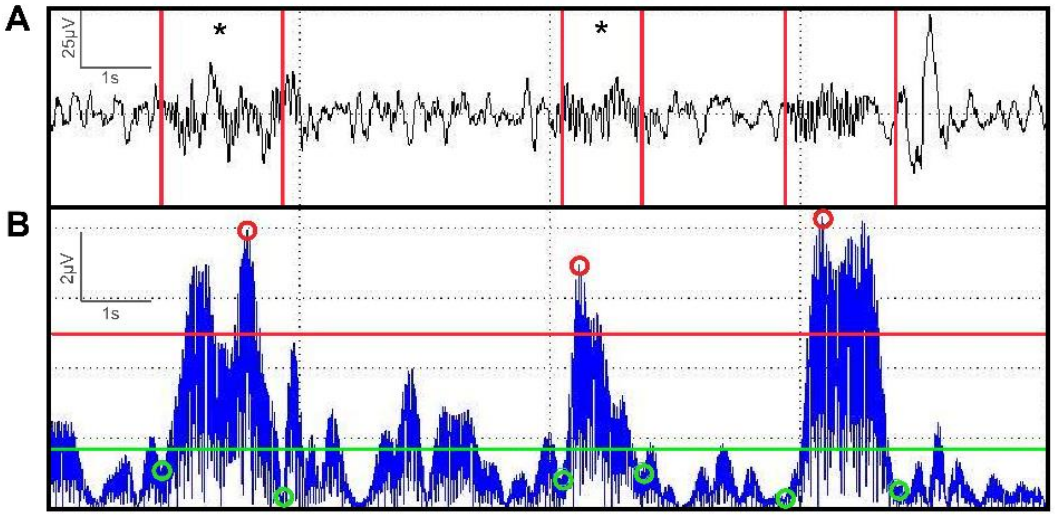

Figure 2. Example of spindle detection for the C3 derivation. **A** raw signal **B** filtered and rectified signal between 12-15 Hz. Vertical red lines enclose detected spindles using an upper threshold of 5 times the mean signal (red line, green line denotes lower threshold). Red circles denote spindle peak amplitude and green circles beginning or end of the sleep spindle. Please note that stars indicate spindles that would not have been detected using a threshold of 8 times the mean signal.

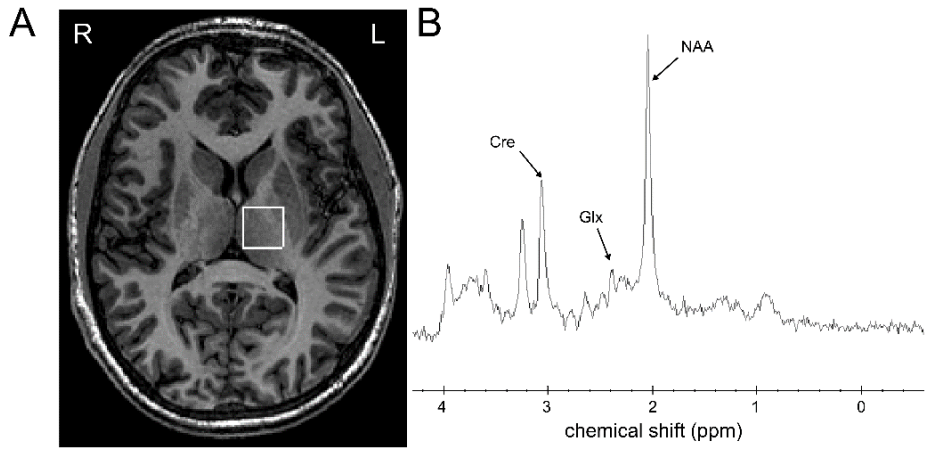

Figure 3. A Placement of the 20x20x20 mm^3 voxel of interest (VOI) on the left thalamus. **B** Illustrative MRS spectrum of a 20-year old healthy subject. Cre, creatine; Glx, glutamine+glutamate; NAA, N-acetylaspartate; L, left; R, right.

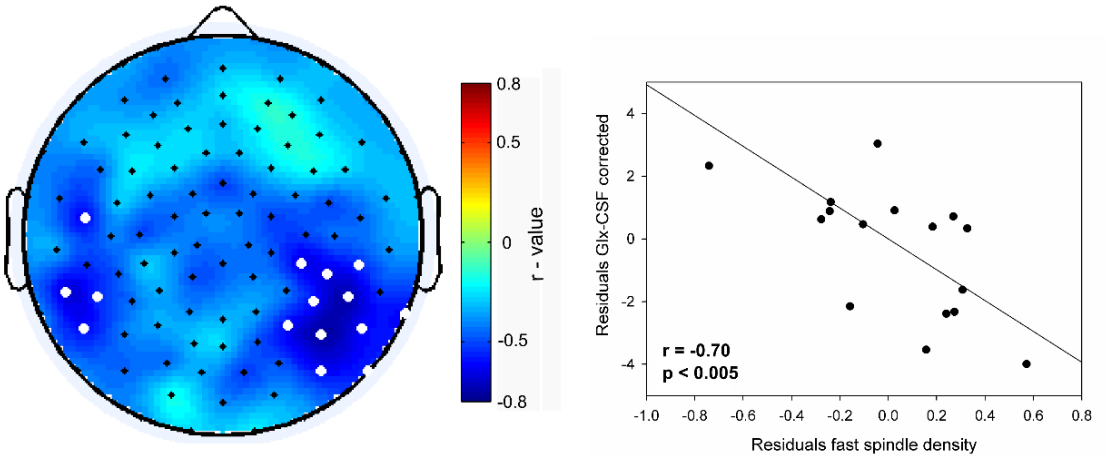

Figure 4. Illustration of the correlation between fast spindle density (14.25-14.75 Hz) and thalamic Glx/Cre levels controlled for CSF and additionally covaried for grey to white matter fraction (GM/WM). Topographical distribution of r-values is plotted on the planar projection of the hemispheric scalp model with negative correlations reflected in blue. White dots indicate significant electrodes ($r > 0.54$, $p < 0.025$). Please note that no correction for multiple testing was applied due to a lack of a good approach to perform a SnPM cluster analysis for a partial correlation design. The scatter plots further demonstrate the significant correlation for the highlighted electrode clusters.

4.4 The Multidimensional Aspects of Sleep Spindles and Their Relationship to Word-Pair Memory Consolidation

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Abstract

Study objectives: Several studies proposed a link between sleep spindles and sleep-dependent memory consolidation in declarative learning tasks. In addition to these state-like aspects of sleep spindles, they have also trait-like characteristics, i.e. were related to general cognitive performance an important distinction that has often been neglected in correlative studies. Furthermore, from the multitude of different sleep spindle measures, often just one specific aspect was analyzed. Thus, we aimed at taking multidimensional aspects of sleep spindles into account when exploring their relationship to word-pair memory consolidation.

Design: Each subject underwent two study nights with all-night high-density EEG recordings. Sleep spindles were automatically detected in all EEG channels. Subjects were trained and tested on a word-pair learning task in the evening, and retested in the morning to assess sleep-related memory consolidation (overnight retention). Trait-like aspects refer to the mean of both nights and state-like aspects were calculated as the difference between night 1 and night 2.

Setting: Sleep laboratory

Participants: 20 healthy male subjects (age: 23.3 ± 2.1 years)

Measurements and Results: Overnight retention was negatively correlated with trait-like aspects of fast sleep spindle density and positively with slow spindle density on a global level. In contrast, state-like aspects were observed for integrated slow spindle activity, which was positively related to the differences in overnight retention in specific regions.

Conclusion Our results demonstrate the importance of a multi-dimensional approach when investigating the relationship between sleep spindles and memory consolidation and thereby provide a more complete picture explaining divergent findings in literature.

Introduction

Numerous behavioural studies link sleep spindles, a unique electrophysiological characteristic of non-rapid eye movement (NREM) sleep, to declarative memory consolidation, thereby claiming that sleep spindles are involved in consolidation processes (for reviews see Fogel and Smith, 2011; Rasch and Born, 2013). A task often used to demonstrate the relationship between sleep spindles and sleep dependent declarative memory consolidation is the verbal associate learning task (word-pair task). Several studies show that sleep-related retention in a word-pair task was positively correlated with sleep spindle activity (e.g. EEG power 12-15 Hz) or density (number of spindles per minute of sleep) (Schabus *et al.*, 2004; Schmidt *et al.*, 2006; Holz *et al.*, 2012; Piosczyk *et al.*, 2013). In addition, an increase of sleep spindle density was found after word-pair learning (Gais *et al.*, 2002), in particular, when the encoding was difficult (Schmidt *et al.*, 2006). This increase of spindle density was correlated with sleep-related changes in recall performance (Schmidt *et al.*, 2006). However, there are also numerous studies which failed to demonstrate positive (e.g., Schabus *et al.*, 2008; Tucker and Fishbein, 2009; Westerberg *et al.*, 2012; Griessenberger *et al.*, 2013) or even negative correlations (Lustenberger *et al.*, 2012) between sleep spindles and memory consolidation were reported using the same learning paradigm.

A possible explanation for such a discrepancy is that sleep spindles have multidimensional aspects, e.g. slow vs. fast frequency spindles (for a review see De Gennaro and Ferrara, 2003) that may serve different functions in memory processes (e.g. (Mander *et al.*, 2011; Cox *et al.*, 2012; Saletin *et al.*, 2013)). In addition, behavioural studies without manipulations can poorly distinguish between the possibility that the relationship between task learning and subsequent sleep spindles is a reflection of general learning abilities during wakefulness or the possibility that sleep spindles play a causal role in learning and memory. In this regard, sleep spindles were proposed to reflect trait- and state-like characteristics (for a review see (De Gennaro and Ferrara, 2003)). Hereby a trait represents a biological fingerprint that is not restricted to specific situations and fairly stable over time (Allport, 1931; Chen *et al.*, 2000). For example, absolute spindle measures per night are trait-like, since they are highly correlated from night to night (Finelli *et al.*, 2001). Trait-like spindle measures were associated with learning and intellectual abilities (Bódizs *et al.*, 2005; Schabus *et al.*, 2006; Fogel *et al.*, 2007a; Fogel and Smith, 2011; Geiger *et al.*, 2011; Geiger *et al.*, 2012; Lustenberger *et al.*, 2012) and sleep related memory consolidation in different learning tasks (e.g., Schabus *et al.*, 2004; Genzel *et al.*, 2009; Tucker and Fishbein, 2009; Holz *et al.*, 2012; Piosczyk *et al.*, 2013). It is difficult, however, to resolve whether performance changes after sleep purely reflect sleep

related consolidation processes or rather mirror a general learning trait (Stickgold, 2004; Schabus *et al.*, 2008; Lustenberger *et al.*, 2012). State-like aspects, on the other hand, are situation specific changes in sleep spindles and may be defined as the differences between nights. Thus, the increase of sleep spindles after learning is a typical state-like aspect of sleep spindles (Schmidt *et al.*, 2006; Schabus *et al.*, 2008). Hence, it was proposed that the inter-individual baseline differences in sleep spindles (trait-like aspects) are correlated with learning potential, whereas learning-related increases in sleep spindles (state-like aspects) reflect processes specific to memory consolidation (Schabus *et al.*, 2008; Fogel and Smith, 2011). However, trait-like and state-like aspects of sleep spindles and their relation to memory consolidation were mostly independently investigated and many studies neglected a differentiation between trait and state-like aspects.

Another issue common to several studies is the selective choice of a specific sleep spindle measure. Thus, from the multitude of different sleep spindle measures, often just one specific aspect was selected (e.g. power or density), in a restricted frequency range (slow vs. fast), for a certain sleep stage (NREM stage 2 vs. SWS) or at a specific time during sleep (early vs. late). Furthermore, analysis was mostly constrained to a few electrodes possibly explaining some divergent findings and confusions in literature. A striking example is the variety of frequency range definitions used for slow and fast sleep spindles (e.g. cut-off frequency 12 Hz (Marshall *et al.*, 2006; Marshall *et al.*, 2011; Ngo *et al.*, 2013b) vs. 14 Hz (Fogel *et al.*, 2007b; Piosczyk *et al.*, 2013)). This discrepancy calls for a more objective way to separate them.

In summary, no study combined all possible spindle measures and assessed topographical differences. Our study aim was to take the multidimensional aspects of sleep spindles into account when exploring trait- and state-like aspects of sleep spindles and their relationship to word-pair memory consolidation. The rational for this study was to increase our understanding of the divergent/confusing findings in literature and thereby providing a more complete and conclusive picture about the relationship between sleep spindles and word-pair learning.

Method

Participants and Design

Twenty young male participants (age: 23.3 ± 2.1 years, mean ± SEM) without sleep disorders, personal or family history of psychopathology, chronic diseases, and current use of psychoactive agents or other medications were recruited. The data used in this manuscript are part of a larger study that was approved by the cantonal ethic commission in Zurich (Switzerland) and for which all subjects gave written informed consent to participate. The study comprised of different conditions, but for this manuscript only baseline nights were analyzed for all subject. We only included male subjects since sleep spindles and sleep related memory consolidation are known to be influenced by the menstrual cycle (Driver *et al.,* 1996; Genzel *et al.*, 2012). Participants were right handed, non-smokers and free of medication and drugs. During a screening night subjects were acclimatized to the lab and the high density EEG (hdEEG) recordings. All included subjects were healthy sleepers with good sleep quality and no sleep disorders were detected. Thereafter, subjects underwent two study nights two weeks apart in the sleep laboratory of the Institute of Pharmacology and Toxicology, University of Zurich. In the evening subjects had to memorize and recall word-pairs in a paired associate learning task. Subsequently high-density EEG (hdEEG) nets were applied and subjects went to bed either at 22:50 or 23:40. Eight hours later (06:50 or 07:40) subjects were woken up and retested on the paired associate learning task. We used the exactly same procedure for both study nights. Three days before these two nights subjects had to adhere to regular bedtimes (8 h time in bed, according to scheduled bedtime in the lab), abstain from caffeine, naps and alcohol. Compliance with the instructions were controlled by breath alcohol test, sleep logs and wrist-worn actometers.

Sleep EEG recordings

A hdEEG (Electrical Geodesics Sensor Net for long-term monitoring, 128 electrodes, including EOG and EMG) net was adjusted to the vertex (Cz) and filled with gel electrolyte. HdEEG provides good spatial resolution thereby allowing the analysis of topographical and local aspects of the sleep EEG (Lustenberger and Huber, 2012). During the 8 hours of continuous sleep EEG recording, the analog signals were referenced to Cz, band-pass filtered (0.01 Hz - 200 Hz), and digitized at 500 Hz. Pre-processing of the signal included filtering (0.5 Hz high-pass, 40 Hz low-pass filter) and down sampling to 128 Hz. Sleep stage scoring was performed on 20-s epochs according to standard criteria (Iber *et al.*, 2007), and artefacts were identified on a 20-s basis by visual inspection and semi-automatically. During the semi-

automatic artefact detection the algorithm automatically excluded epochs with a power value exceeding 13 times the power of the sliding mean (average over 15 20-s epochs) in the 0.75–4.5 Hz frequency band and a power value exceeding 6 times the power of the sliding mean (average over 25 20-s epochs) in the 20–30 Hz frequency band. Please note, that we used a user interface that enabled us to further exclude epochs that were clearly deviant from the background by manually adjusting the threshold.

In a next step, the EEG was re-referenced to average reference after exclusion of EEG channels of insufficient quality (on average, 7 channels per subjects). For topographical analysis of sleep-EEG activities ("topoplots", figure 4) bad channels were interpolated using a spherical interpolation provided by the EEGLAB toolbox (Delorme and Makeig, 2004). We only included 108 channels into the statistical analysis (excluding marginal electrodes which would lead to 128), but used interpolated values of bad channels that belonged the 108 channel configuration.

Spindle analysis

To address the multidimensional aspects of sleep spindles we included all electrodes of the hdEEG. As a consequence of including >100 EEG channels, a visual detection of sleep spindles was not feasible and we automatically detected sleep spindles for each electrode using an established algorithm (Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010). Detailed description about this procedure can be obtained from Ferrarelli et al. (2007). Specifically, the signal was band-pass filtered between 12-15 Hz and rectified. A sleep spindle was detected from the signal if the amplitude exceeded an upper threshold that was defined relative to the mean signal amplitude (8 times mean signal). Beginning and end of sleep spindles were defined as the time points when the signal around the peak amplitude dropped below a lower threshold (2 times mean signal). In order to optimize the analysis for our specific aim we adapted this original procedure as follows. Since it was important for our study to accurately distinguish between slow and fast spindle frequencies we needed to adapt the frequency range. As shown by preliminary analysis, the originally used 12-15 Hz band-pass filter provided best signal-to-noise ratios, but was too narrow in order to accurately detect slow spindles (around 12Hz and lower). It is well established that sleep spindles exist within the frequency range of 10-16 Hz (e.g. (Gibbs and Gibbs, 1950; Andrillon *et al.*, 2011; Warby *et al.*, 2014); please also see supplemental Figure 1 for spectral plots). Previous studies also used band-pass filter settings between 10-16 Hz to investigate spindles (e.g. (Peter-Derex *et al.*, 2012)). Thus, to detect sleep spindles in this broader frequency range, we used a Chebychev filter of order 20 with nominal

passband corner frequencies at 10 and 16 Hz and stopband corner frequencies at 5 Hz (at least 80 dB dampening ≤ 5 Hz) and 32 Hz (at least 80 dB dampening ≥ 32 Hz) allowing an accurate and negligible dampening around 11-16 Hz (empirically determined dampening at 11 Hz was 1.9 % and 0.1 % at 16 Hz). Since this broadening of the filter-band decreased the signal-to-noise ratio, we had to adapt the upper and lower threshold accordingly. An upper threshold of 5 times and a lower threshold of 1.25 times the mean signal provided best spindle detection as verified by visual inspection. We used channel-wise threshold definition because signal amplitude varied significantly between channels. In addition this approach aims at taking factors into account that might vary across channels (e.g. skull thickness) and would influence EEG signal amplitude. Besides using channel-wise threshold approaches there are also multi-channel studies which found good results using one common threshold for all channels at least in analysis where spindle detections were performed separately for slow and fast spindles (Mölle *et al.,* 2011). Further studies are needed to investigate whether both approaches work equally well and provide comparable results of detected sleep spindles. Please note that a further broadening of the filter settings would lead to high noise, i.e. no spindle specific activity (e.g. alpha activity in the low spindle frequency range), and as a result spindle detection becomes inaccurate. Using this adapted algorithm our spindle density values were in the range of other studies using visual and automatic spindle detection (e.g., Dijk *et al.*, 1993; Nicolas *et al.*, 2001). Moreover, they also showed the expected topographical distribution (see supplemental Figure 2 for the topographic distribution of slow and fast spindles in the supplement). Our algorithm provides different spindle measures and we focused on sleep spindle density (number/min), which has often been related to declarative memory consolidation (Schabus *et al.*, 2004; Schmidt *et al.*, 2006; Rasch and Born, 2013), and the averaged integrated spindle activity of individual sleep spindles (µVs), which provides information about the shape or the "power" of the sleep spindles. The integrated spindle activity comprises of spindle amplitude and duration and is calculated as follows: The band-pass filtered absolute EEG signal is first summed up over the course of each sleep spindle and then averaged for all included spindles. Both sleep spindle measures are quantified for different frequency bins from 11-16 Hz (spindles were detectable in this frequency range, see supplemental Figure 3 with a histogram of the spindle distribution). We used the "*histc"* function provided in MATLAB to divide the sleep spindles in to specific frequency bins with a frequency resolution of 0.5 Hz (e.g. the 10-Hz bin covers all the spindles that are >10 Hz and <10.5 Hz).

Furthermore, sleep spindles with a duration lower than 300 ms were excluded (Warby *et al.*, 2014). This analysis was performed for the first (FH) and the last hour (LH) of artifact

free NREM sleep. By focusing on FH and LH of NREM sleep, we included the timing of the sleep spindle during the sleep period and also accounted for different levels of sleep pressure (FH: high sleep pressure, in particular sleep stage N3; LH: Low sleep pressure, in particular sleep stage N2).

Word-pair task

Based on several earlier studies, the paired associate learning or word-pair task was used to assess sleep dependent performance improvement in declarative memory (Gais *et al.*, 2002; Clemens *et al.*, 2005; Marshall *et al.*, 2006; Genzel *et al.*, 2012; Ngo *et al.*, 2013b). Forty semantically related word-pairs were presented on a computer screen for 4 s each, separated by an inter-stimulus interval of 100 ms. After the presentation, there was an immediate cued-recall, where the first words of the word-pairs were presented in random order and the second one had to be recalled. The subjects were asked to guess in case they did not remember the word. No time limit for the answers was set and once the subjects entered their answer, a feedback for accuracy was provided and the correct word-pair was presented again for two seconds. The subjects were instructed to memorize the word-pair again during the feedback. In the morning (45 minutes after subjects woke up), there was a delayed recall, where the procedure was the same as in the immediate recall. Overnight retention was defined as the difference in correct answers between delayed (morning) and immediate recall (evening). It is important to mention that our retention measure does not allow us to differentiate between a sleep-dependent memory consolidation and learning related improvement due to feedback that was given during the immediate recall as done in many previous studies (Marshall *et al.*, 2006; Rasch *et al.*, 2009; Wilhelm *et al.*, 2011; Genzel *et al.*, 2012; Lustenberger *et al.*, 2012; Payne *et al.*, 2012; Griessenberger *et al.*, 2013; Ngo *et al.*, 2013b). We used two parallel lists for the two nights in a randomized order.

Statistics

First, we compared the results of the word-pair task between night 1 and night 2 using paired t-tests and bivariate Pearson correlations. In order to investigate trait- and state-like aspects of sleep spindles and their association to overnight retention, we averaged our measures for both nights (trait) or took the difference between night 1 and night 2 (state). For both trait and state, Pearson correlation matrices between sleep-dependent overnight changes of a word-pair task and spindle measures (density, integrated spindle activity) for each frequency bin (11-16 Hz, 0.5 Hz bins) and all electrodes (108, marginal electrodes were excluded, q.v. grey filled electrodes in Figure 5) were performed. For correlations with trait-like aspects, we further controlled for evening performance in a partial correlation design (see results for more details). For both trait- and state-like aspects we conducted hierarchical cluster analysis on the *r*-values of the two dimensional correlation matrices (channels/electrodes x frequency bins). We used this exploratory approach to elaborate in a objective way the frequency bins that show similar correlations with the word-pair variables for multiple EEG channels (clustering of a 2D correlation matrix of frequency bins x channels) and are clearly separated from other frequencies. To do so, a 2D hierarchical cluster analysis of the Euclidean distances between all the 11 frequency bins and all 109 EEG channels was performed based on their correlation (r-values) with the word-pair task using the "clustergram" function of the bioinformatics toolbox provided by MATLAB. The 2D correlation matrix is grouped into a hierarchical cluster tree (dendrogram) according to their proximity or how close the correlation coefficients are. A dendrogram consists of upside-down U-shaped lines or branches that are also called clades. Each terminal end of the clade is called leave (with represent frequency bins on the x-axis and channels on the y-axis in our examples). The arrangement of the clades indicates which frequency bins and channels (leaves) are most similar to each other. The height of the branch/clade indicates how similar or different they are from each other: the smaller the height, the closer correlation coefficients are for the clustered frequency bins and channels. To calculate this height or distance between the clusters, the "single linkage method" (nearest neighbor defined as the smallest Euclidean distance: shortest connection line between 2 points) was applied. To define which similarity in correlation coefficients is useful to define frequency clusters in our data set, we performed visual inspection of the derived dendrograms and tested different Euclidean distances for the definition of clusters. Using 3.5, which is an arbitrary cut-off derived from visual inspection, the derived frequency clusters were the most meaningful ones across all dendrograms.

An illustrative example of such a two-dimensional dendrogram is shown in Figure 3. Subsequently, neighboring frequency bins that clustered together (below Euclidean distance of 3.5) were averaged (integrated spindle activity) or summed up (density). Please note, that the cluster analysis included all *r*-values and not just the significant ones. In order to control for multiple comparison we only included frequency bands that contained at least more than 5% (number of electrodes x included frequency bins above the chance level) of significant correlations ($p<0.05$). In a next step, we tested for significant electrode clusters by calculating the correlation between the chosen frequency bands of sleep spindle measures and sleep-related performance changes in the word-pair task. This topographical analysis provides further information about the spatial clustering (channel clustering instead of frequency bin clustering) of the correlations between sleep spindle measures and word-pair retention. To control for multiple comparisons and to define specific regions of interest (Nichols and Holmes, 2002; Huber *et al.*, 2004; Ferrarelli *et al.*, 2007; Ringli *et al.*, 2013) we applied statistical nonparametric mapping (SnPM (Nichols and Holmes, 2002)) whenever appropriate. A supra-threshold cluster analysis was applied in which electrodes that were above/below a correlation coefficient of 0.39/-0.39 (corresponding to a *p*-value of 0.1 for $n = 19$) and exceeded the 90th percentile cluster size given by the permutation analysis were considered for further analysis.

One subject had to be excluded from the whole analysis due to bad sleep and data quality (movement artifacts, many bad channels).

Results

Sleep quality

All included subjects ($n=19$) had good sleep quality in the first and second experimental night (Table 1). During night 1 subjects had a significantly longer sleep latency, more NREM stage 1 (N1) sleep and less REM sleep (Table 1). When both nights are averaged, the first hour of NREM sleep (FH_{NREMS}) includes significantly more N3 sleep (31.1 ± 2.0 vs. 6.1 ± 1.3 min) and significantly less N2 sleep (28.9 ± 2.0 vs. 53.9 ± 1.3 min) compared to the last hour of NREM sleep (LH_{NREMS}).

Table 1. Sleep variables derived from visual scoring.

Sleep variable	Night 1 Mean (SEM)	Night 2 Mean (SEM)	Difference P-value
Total sleep time (min)	453.7 (3.7)	458.3 (3.5)	>0.1
Sleep efficiency (%)	94.8 (3.2)	95.7 (3.1)	>0.1
Sleep latency (min)	17.3 (1.6)	9.2 (1.3)	<0.001
Wake after sleep onset (min)	12.8 (2.9)	14.0 (3.4)	>0.1
N1 (min)	26.8 (2.1)	19.5 (2.1)	<0.01
N2 (min)	239.6 (5.8)	242.7 (7.8)	>0.1
N3 (min)	79.4 (5.7)	80.1 (6.8)	>0.1
REMS (min)	107.8 (4.1)	116.0 (4.6)	<0.05

Time in bed was 8h. Sleep latency: duration until the first occurrence of sleep stage 2. N1-3: NREM sleep stage 1-3. REMS: Rapid eye movement sleep.

Word-pair task

A summary of the performance in the associate verbal learning task is presented in Figure 1. Subjects recalled significantly more word-pairs in the delayed recall (morning) compared to the immediate recall (evening) in both nights ($p<0.05$). This significant overnight improvement is expected since feedback was given during the immediate recall in the evening allowing the subjects to re-encode the word-pairs (Lustenberger *et al.*, 2012). Immediate recall was positively correlated between the two nights ($r_{19} = 0.51$, $p = 0.02$). Overnight retention (difference morning to evening) also tended to be correlated between the two nights ($r_{19} = 0.40$, $p = 0.09$). These data indicate that immediate recall and overnight retention are partially reproducible. Thus, the average of the two nights likely represents a trait aspect. Number of recalled word-pairs in the immediate and delayed recall was not different between the two nights (Figure 1A) whereas overnight retention was significantly higher in the first compared to the second night (Figure 1B). Thus, the difference between night 1 and night 2 can be used as a state measure for the overnight retention.

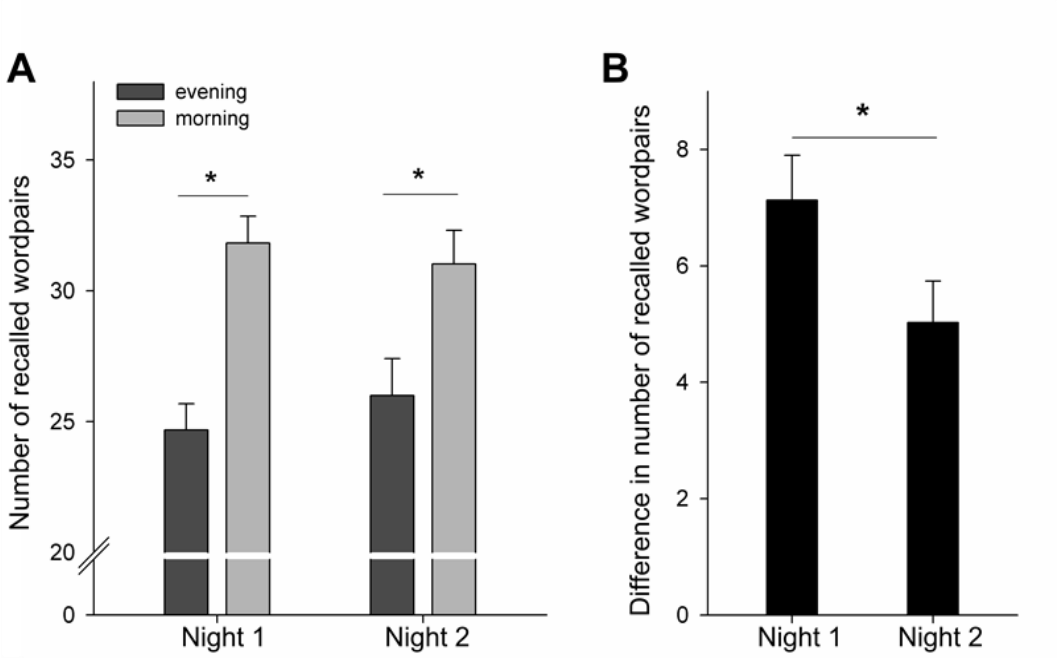


Figure 1. A Recall performance of associate verbal learning task of both nights. Stars indicate significant differences ($p < 0.05$, paired *t*-test, $n=19$) between evening and morning recall. B Overnight retention was also significantly different between the two nights as indicated by a star ($p < 0.05$, paired *t*-test, $n=19$).

Importantly, when both nights are averaged (trait), evening performance tended to negatively correlate with overnight retention ($r_{19} = -0.46$, $p = 0.05$, we also found a tendency when both nights were analyzed separately; Night 1: $r_{19} = -0.42$, $p = 0.08$; Night 2: $r_{19} = -0.40$, $p = 0.09$). This was not the case for the state (night difference) variables ($r_{19} = -0.26$, $p > 0.25$). Thus, we included the evening performance as a covariate (correlation of residuals that are controlled for evening performance) in the Pearson correlations that focus on trait-like aspects of sleep spindles (FH and LH) and word-pair learning using a partial correlation design.

Trait and state aspects of sleep spindles and their association to overnight retention in a word-pair task

Correlation matrices between overnight retention and the two sleep spindle measures (density, integrated spindle activity) for trait/state and FH_{NREMS}/LH_{NREMS} are illustrated in Figure 2. These matrices include all 108 electrodes of the hdEEG net and frequency bins from 11-16 Hz (0.5 Hz resolution). Based on these correlation matrices we performed a hierarchical cluster analysis (see Methods for details). An illustrative example of such a two dimensional hierarchical tree (dendrogram) is provided in Figure 3. All neighboring frequency bins that grouped together as a cluster in the dendrogram (Euclidean distance < 3.5) and had significant electrodes above the chance level (number of significant correlations > 5% of electrodes x frequency bins) were used for topographical plots.

When looking at trait aspects, we found that during the first hour of NREM sleep fast sleep spindle density was inversely correlated with overnight retention (controlled for evening performance) on a rather global level (Figures 2 and 4). Conversely, during the last hour of NREM sleep, slow sleep spindle density was positively related to overnight retention (also controlled for evening performance).

State-like aspects (difference between the two nights) are illustrated in the second row of Figure 4. This analysis shows that slow sleep spindle measures were positively related to overnight retention, generally on a spatially more local level. To identify these local clusters of electrodes we performed statistical non-parametric mapping (q.v. methods). The significant clusters are highlighted with blue circles. Our analysis of state-like aspects revealed a cluster of frontal electrodes for the density of slow spindles and a cluster of left centro-parietal and right temporo-occipital electrodes for integrated activity of slow spindles. A comparison of these clusters between the first and second night revealed that only the electrode clusters of slow integrated spindle activity in LH_{NREMS} were significantly higher in night 1 compared to night 2 in subjects that showed superior positive overnight retention in night 1 compared to night 2 (Figure 5).

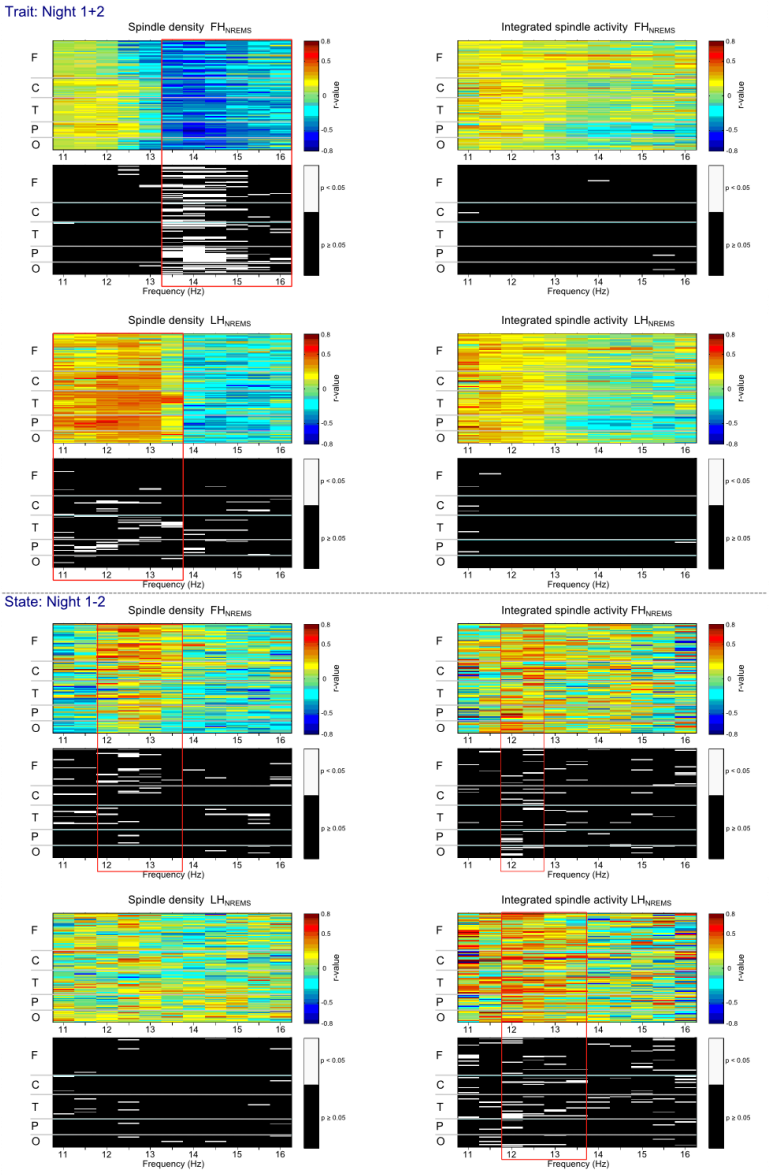


Figure 2. Heat plots of the correlation coefficients and corresponding statistics for the relationship between spindle measures (density [number/min] and integrated spindle activity [μVs]) of the first (FH) and last hour (LH) of NREM sleep and overnight retention in word-pair learning for trait- (average of night 1 and 2) and state-like (difference between nights) aspects. In each plot the x-axis represents spindle frequency and the y-axis the 108 high density EEG electrodes grouped into sets of electrodes that were close in distance to the 10-20 system configuration. F: Frontal, C: Central, P: Parietal, T: Temporal and O: Occipital. Red rectangles highlight neighboring frequency bins that were grouped together in a hierarchical cluster analysis (see Figure 3) and include number of significant correlations above the chance level (see methods, white bars, $p<0.05$).

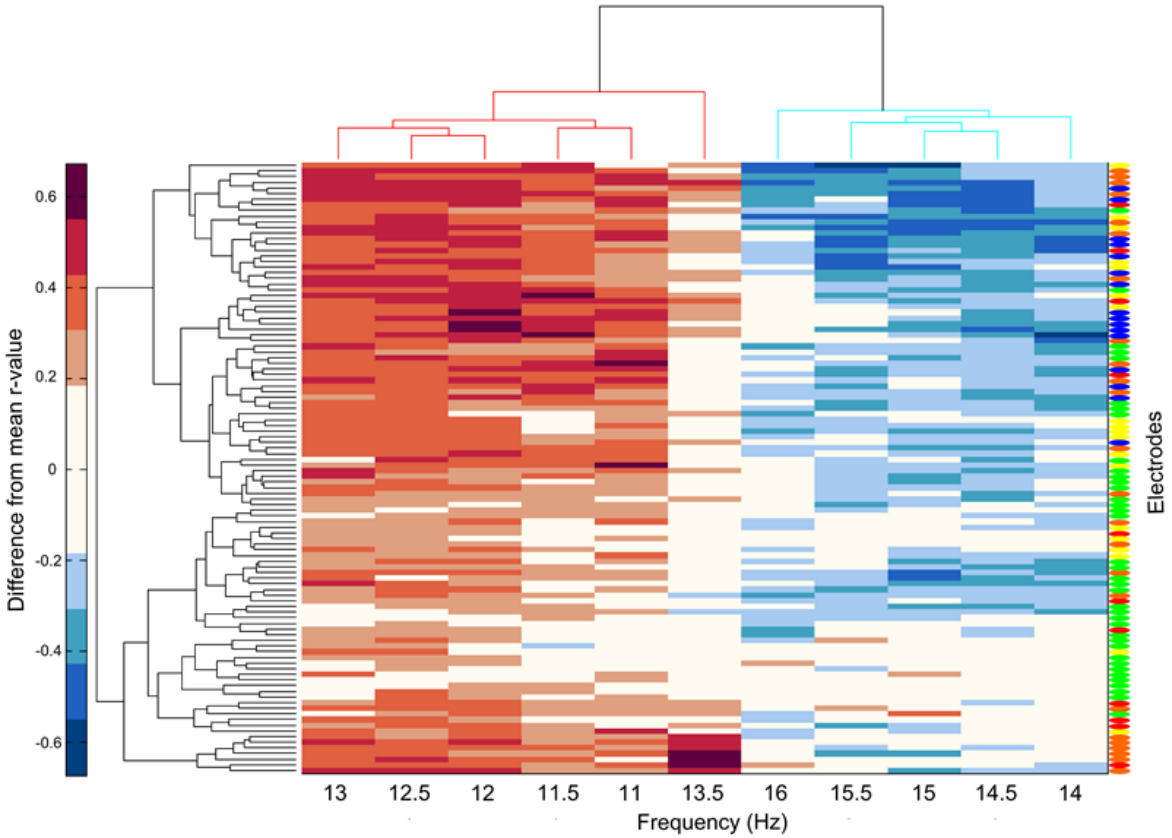


Figure 3. Illustrative example of the two dimensional hierarchical cluster trees (dendrogramm) and the heat plot of the *r*-values of a partial correlation between trait-like sleep spindle density during the last hour of NREM sleep and overnight retention, controlled for immediate recall performance. Please note that colors of the heatplot encode differences from the mean *r*-value, with blue colors indicating lower and red higher values than the mean. Red/blue marked branches illustrate clusters with an Euclidean distance below 3.5. A clear differentiation between slow and fast spindle density is observable with a cut-off frequency at 13.5 Hz. Color coded ellipses are used to code for the electrode location on the scalp: green-frontal, yellow-central, red-occipital, orange-temporal, blue-parietal.

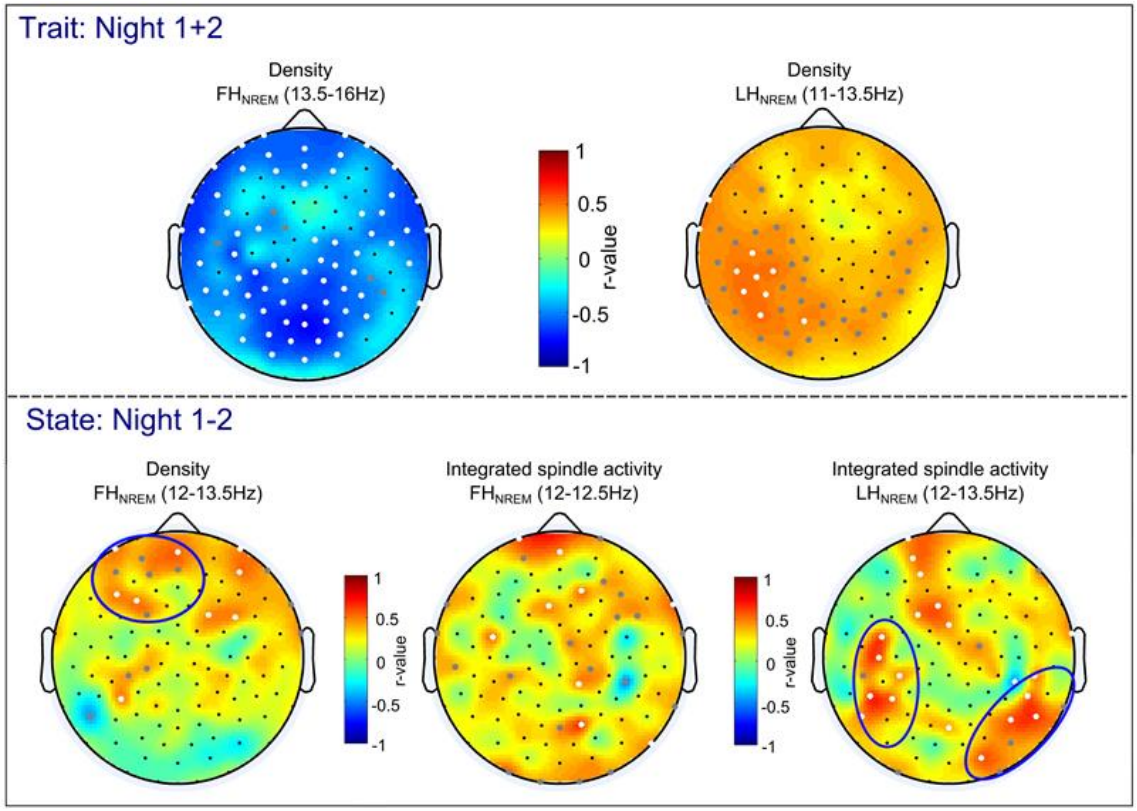


Figure 4. Top panel: Topographical representation of the correlation coefficients highlighted by red rectangles in Figure 2 (trait aspect; spindle density). White dots indicate electrodes with significant correlations (p<0.05, partial Pearson correlation controlled for evening performance) and grey dots indicate trend level partial correlations (p<0.1). **Bottom panel:** Reported correlation coefficients are based on a Pearson correlation (not controlled for evening performance difference, white dots $p < 0.05$, grey dots $p < 0.1$). Clusters (state aspect) that survived statistical non-parametric mapping (SnPM $p < 0.1$) are highlighted with blue circles.

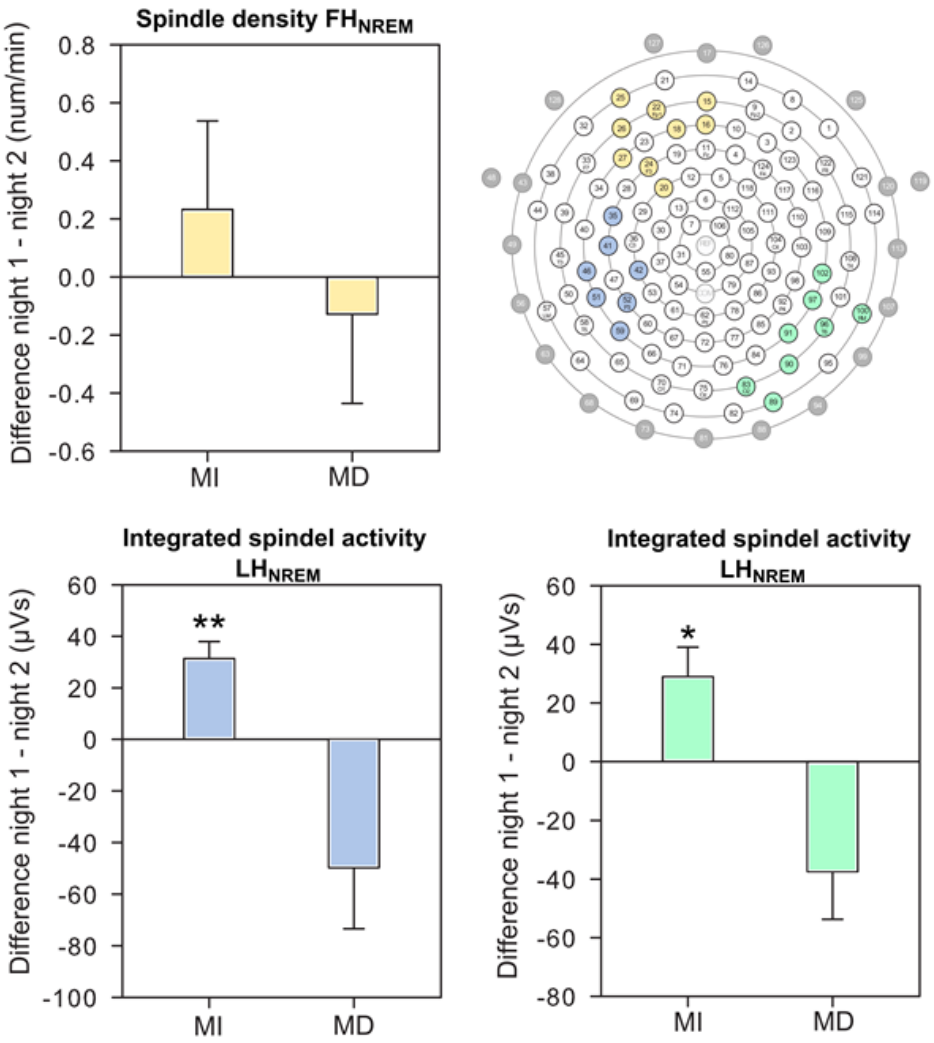


Figure 5. Difference of spindle density (number/min) and integrated spindle activity (µVs) between night 1 and night 2 for clusters that survived SnPM correction (Figure 4, 12-13.5Hz). Electrode clusters are color coded in the topographical outline of the high density EEG net. Individual differences were divided into two groups according to their word-pair retention performance; MI: Higher overnight retention rate during night 1 than night 2 (n = 15); MD: Lower retention rate during night 1 than night 2 (n = 4). Stars indicate significant differences between night 1 and night 2 (paired t-test, *<0.05, ** <0.01). Please note that due to a low number of subjects no statistical analysis was performed for the MD group. However, in the MD group all 4 subjects showed reduced integrated spindle activity in the two clusters and three of 4 subjects had reduced spindle density. Grey filled marginal electrodes were not included in the analysis.

Discussion

Our results demonstrate the importance of a multi-dimensional approach when investigating the relationship between sleep spindles and memory consolidation. We found different associations between memory retention and spindle measures, depending on the type of the spindle measure, spatial location and time of night. In addition, the differentiation between trait- and state-like aspects of sleep spindles and their relation to declarative verbal associate memory adds to the picture. Our approach shows the complexity of the relationship between sleep spindles and declarative memory and thereby provides a more conclusive picture that might explain the divergent findings reported in literature.

Trait-like aspects of sleep spindles and overnight retention

Most of the studies that performed correlations between sleep spindles and overnight/nap retention in word-pair learning focussed on trait-like aspects of spindles. These studies found controversial results with opposite signs of the correlations (Schabus *et al.*, 2008; Tucker and Fishbein, 2009; Lustenberger *et al.*, 2012; Westerberg *et al.*, 2012; Griessenberger *et al.*, 2013). Our analyses showed a similar discrepancy. Most of the studies reported positive correlations of overnight/nap retention in word-pair learning with spindle density (Genzel *et al.*, 2009; Mednick *et al.*, 2013), sigma activity (Holz *et al.*, 2012; Piosczyk *et al.*, 2013), peak amplitude (Ngo *et al.*, 2013b) or a combination of these measures (Schabus *et al.*, 2004; Genzel *et al.*, 2009). Interestingly, these positive associations were mainly restricted to stage 2 sleep (Genzel *et al.*, 2009; Holz *et al.*, 2012; Mednick *et al.*, 2013; Piosczyk *et al.*, 2013) and/or spindle frequencies between (12-14Hz, Holz *et al.*, 2012; Piosczyk *et al.*, 2013). In line with these observations, we found a positive correlation between trait-like slow spindle density (11-13.5 Hz) in the last hour of NREM sleep, a time window that is dominated by stage 2 sleep, and overnight retention. Notwithstanding, other studies found no correlation between trait-like spindle measures and overnight memory retention (e.g., Schabus *et al.*, 2008; Tucker and Fishbein, 2009; Westerberg *et al.*, 2012; Griessenberger *et al.*, 2013). These findings can be explained by our results. Our analysis showed contradictory correlations when including different frequency ranges and different time windows. Moreover, we found no significant correlations with integrated spindle activity. Studies often averaged spindle measures for the whole night and frequency range, which may have resulted in a nullification of the correlations.

One of our main results was a negative correlation between overnight retention and fast spindle density in the first hour of NREM sleep. We reported a similar negative correlation

already in an earlier study of our group (Lustenberger *et al.*, 2012). In this study with young adults, we observed that sigma activity (comprising of both, density and integrated spindle activity) was rather related to learning efficiency than sleep specific consolidation. In other words, subjects that encoded much of their capacity (maximal amount of correct word-pairs reached) in a first place (immediate recall performance relative to delayed performance) had more sigma activity and less overnight improvement (Lustenberger *et al.*, 2012). This interpretation raises an important question: What do trait-like spindle measures and overnight retention reflect? Our and previous results indicate that these measures may reflect learning ability per se rather than sleep specific consolidation processes (Schabus *et al.*, 2008; Fogel and Smith, 2011). In line with this interpretation have trait-like aspects of sleep spindles been related to learning and cognitive abilities (Bódizs *et al.*, 2005; Schabus *et al.*, 2006; Fogel *et al.*, 2007a; Fogel and Smith, 2011; Geiger *et al.*, 2011; Geiger *et al.*, 2012; Lustenberger *et al.*, 2012). In addition, word-pair performance improvement/stabilization (retention) after a period of sleep partially depends on the feedback given during the immediate recall (second learning opportunity (Lustenberger *et al.*, 2012)) or the strength of encoding that might then be reflected in sleep spindle measures. Thus, divergent findings may be a result of the design of the word-pair task that substantially differs between the studies in terms of feedback, difficulty of word-pairs, number of encoding opportunities, and the degree of semantic relation. Importantly, in our study, evening performance was negatively related to overnight retention and was included in the correlation as a confounding factor. Since there is good evidence that encoding performance is also related to sleep spindles (Gais *et al.*, 2002; Berner *et al.*, 2006; Schabus *et al.*, 2008), future studies should carefully control for the contribution of this initial performance.

Significant correlations between trait-like aspects of word-pair overnight retention and sleep spindles were restricted to the spindle density measure. In addition, we found a clear, objective cut-off in the direction of the correlations at 13 Hz (FH_{NREMS}) and 13.5 Hz (LH_{NREMS}) separating our slow and fast spindles. Thus, we found significant correlations for the FH of NREM sleep between the frequencies 10-13 Hz and 13.5-16 Hz ($r_{19} = -0.57$, $p < 0.01$), and also the LH tended to be negatively correlated between 11-13.5 Hz and 14-16 Hz ($r_{19} = -0.45$, $p = 0.05$). Why these frequency bands revealed correlation coefficients with opposite directions is not evident. It was hypothesized that these two types of sleep spindles serve different functions (for a review see De Gennaro and Ferrara, 2003) and might result from two different generators - for example from two different thalamic sources (Anderer *et al.*, 2001). However, different spindle frequencies might result from the hyperpolarization-rebound sequence duration of thalamo-cortical neurons (Steriade, 2003) or their level of hyperpolarization (Andrillon *et al.*,

2011). We found a negative correlation between the fast and slow sleep spindle density indicating that they might be related and derive from a single mechanism, as has previously been hypothesized, e.g. in intra-cranial measures of human subjects (Andrillon *et al.*, 2011; Nir *et al.*, 2011). Hence, one might speculate that the duration of hyperpolarization-rebound sequences in the thalamo-cortical neurons or their level of hyperpolarization (either leading to slow or fast spindles) could be related to overnight retention or learning abilities.

State-like aspects of sleep spindles and overnight retention

Even though we had the same study design for both nights, overnight retention in the first night was significantly higher than in the second night while evening performance (immediate recall) was similar. The difference in overnight retention was also not related to the difference in immediate recall. This difference provided an important state-like aspect of declarative overnight retention that might be specifically related to consolidation processes during sleep rather than encoding per se. The causal role of sleep spindles can only be established using selective manipulation of sleep spindles, which is to date not possible. To overcome this problem, we need in a first step different approaches that investigate the role of sleep spindles in memory consolidation in more detail. A fruitful approach to investigate the importance of sleep spindles in memory consolidation is the comparison of learning and non-learning conditions where an increase of spindle density was found in a learning compared to a non-learning condition (Gais *et al.*, 2002). To overcome the limitation of such an approach that alterations in sleep spindles might also be associated with task performance related general plasticity changes in the evening, such trait-like effects could be eliminated by using a within subject design. We applied an alternative approach taking into account a similar evening baseline performance and just comparing nights that had a difference in overnight changes (declarative memory retention) rather than evening learning performance. Thus, our experimental design represents an alternative approach not comparing learning vs. non-learning but focusing on more vs. less declarative memory retention that reflects memory consolidation. If indeed sleep spindles are causally involved in memory consolidation processes we expect that day-to-day variances of declarative memory retention results in different amounts/shapes of sleep spindles, or vice versa, that fluctuations in sleep spindles should be related to differences in overnight retention measures. We therefore addressed the question, whether this difference in overnight retention is related to state-like aspects of sleep spindles. It had been proposed that learning related increases in sleep spindles reflect processes specific to memory consolidation (Schabus *et al.*, 2008; Fogel and Smith, 2011). We found significant positive

correlations between overnight retention and slow sleep spindle density as well as integrated spindle activity in the first and last hour of NREM sleep, respectively. These correlations were restricted to specific regions, frontal for spindle density, and centro-parietal and parieto-temporal for integrated spindle activity. A few studies showed similarly that learning related difference in spindle activity (related to integrated spindle activity (Schmidt *et al.*, 2006; Schabus *et al.*, 2008)) during stage 2 were positively correlated with overnight retention. If indeed sleep spindles are causally involved in declarative memory consolidation processes one would expect higher spindle measures in night 1 compared to night 2. We found such increased spindle measures in night 1 compared to night 2 but only for slow integrated spindle activity and only in subjects that showed superior overnight retention in night 1. Interestingly, the four subjects that had worse overnight retention in night 1 compared to night 2 had all lower integrated spindle activity in night 1. Our results provide a first hint that especially state-like slow integrated spindle activity is localized to specific regions (e.g. left centro-parietal and right temporo-occipital), which might be involved in memory consolidation processes. However, since this study and most others are only correlative in nature we cannot conclude that integrated sleep spindle activity is causally involved in memory consolidation. To date, only a few studies enhanced/reduced spindle measures by manipulation using electrical brain stimulation (Marshall *et al.*, 2006; Marshall *et al.*, 2011), tones (Ngo *et al.*, 2013b) or pharmacological interventions (Feld *et al.*, 2013; Kaestner *et al.*, 2013; Mednick *et al.*, 2013) and showed superior/reduced declarative memory consolidation. However, these studies not only affected sleep spindles, but also modulated slow wave activity (Marshall *et al.*, 2004; Marshall *et al.*, 2006; Feld *et al.*, 2013; Ngo *et al.*, 2013b), slow wave sleep (Mednick *et al.*, 2013) or affected REM sleep (Mednick *et al.*, 2013). Thus, proof of a causal role of sleep spindles in memory consolidation is still missing.

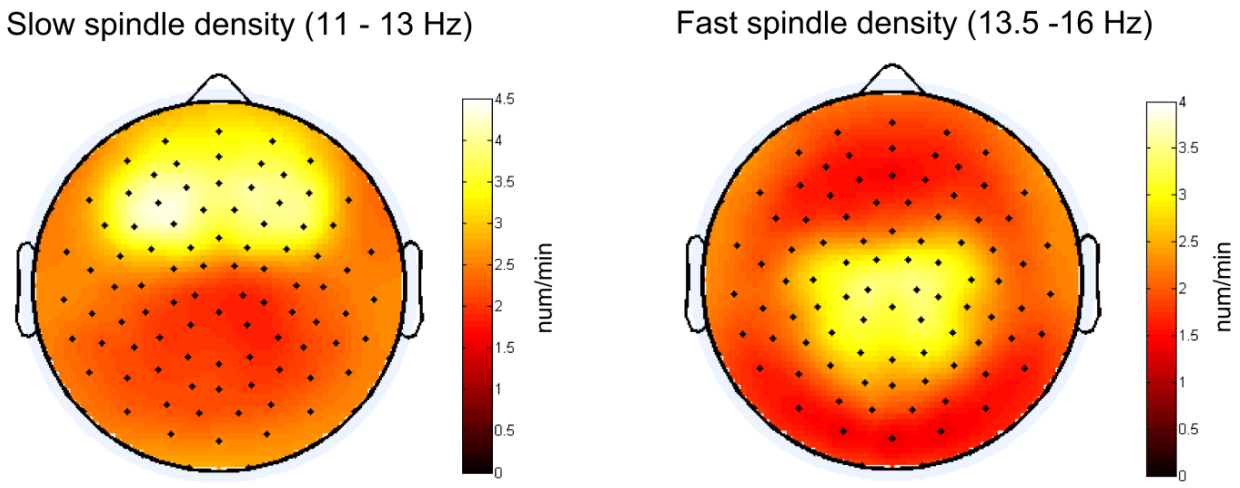
Compared to other studies using single or few electrodes, our hdEEG recordings revealed regional differences, in particular for state-like aspects of sleep spindles. Why our correlations were restricted to these specific regions is unclear. FMRI and PET studies reported different frontal and posterior parts involved in word-pair retrieval and encoding, (e.g. Schmidt *et al.*, 2002). However, interpretation of these regions is precarious, since we cannot directly educe underlying source of spindle activity from cortical EEG topography, especially since thalamo-cortical circuits are involved in their generation.

Finally, it is important to emphasize that we used a specific automated spindle detection algorithm since a visual detection of sleep spindles is not feasible with hdEEG. This might be

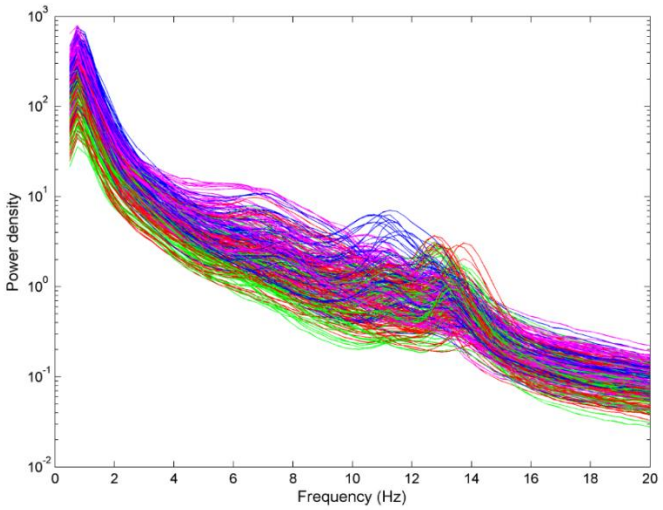
a limitation of our study because it was recently reported that diverging results were obtained with different spindle detection algorithms and visual scoring (Warby *et al.*, 2014). For instance, our filter settings did not allow accurate (very low frequency) spindle detection below 11 Hz due to signal attenuation. However, lowering the low frequency cut-off would also lead to a reduced signal-to-noise ratio since frequencies in the alpha and theta range would also significantly contribute to the filtered signal. Future studies will need to establish and compare valid algorithms to address the multidimensional aspects of sleep spindles. Thus, our multidimensional approach should further be extended including different algorithms as an additional level in the analysis.

In conclusion, our results favour a multidimensional approach in analysing sleep spindles and their relation to memory consolidation. We found clear differences in the association between spindles and word-pair memory consolidation depending on the type of spindle measure, frequency, timing and localization we analyzed. Our results also show that trait-like sleep spindle density reflects learning traits rather than sleep dependent consolidation processes per se. On the other hand, state-like aspects of slow integrated sleep spindle activity might be involved in memory consolidation processes. Tools allowing to selectively manipulating sleep spindles are needed for establishing a causal relationship.

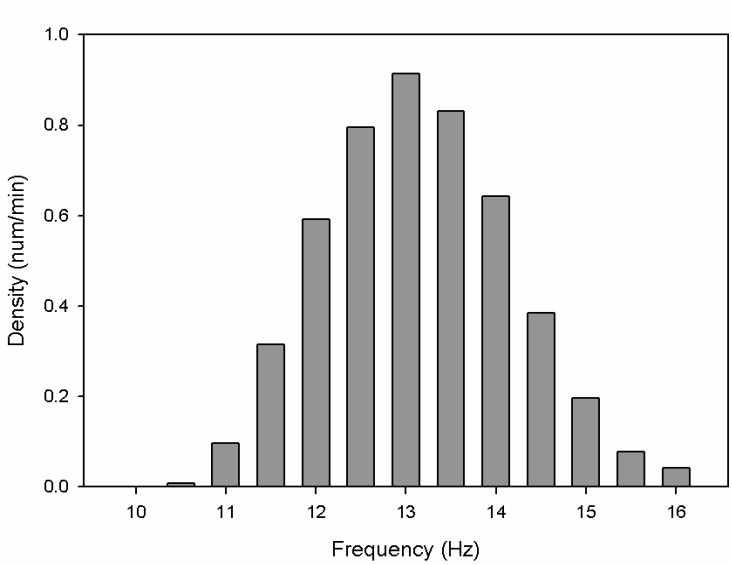
Supplementary



Supplemental Figure 1. Topographical distribution (n = 19) of slow (11 – 13 Hz) and fast (13.5-16 Hz) spindle density averaged for both experimental nights. Topography is based on NREM sleep stages N2 and N3 of the entire night. Values are color coded between 0 and the maximum and plotted on the planar projection of the hemispheric scalp model. Electrode locations are indicated by black dots.



Supplemental Figure 2. Individual power density spectra (power density in $\mu V^2/Hz$) for frequency bins between 0.5 – 20 Hz of 19 subjects, both experimental nights and different electrodes. Blue lines indicate frontal electrodes (F3, F4), green lines central electrodes (C3, C4), red lines parietal electrodes (P3, P4) and magenta lines occipital electrodes (O1, O2).



Supplemental Figure 3. Distribution of spindle densities for 0.5 Hz frequency bins between 10 and 16 Hz. Spindle densities (number/min) were averaged over all 108 electrodes, subjects and experimental nights. Please note that below 11 Hz almost no spindles were detected and that at 16 Hz a clear drop is visible. This limited detection at the boarders is likely caused by the filter (10-16 Hz) that prevented accurate detection close to the cut-off frequencies.

4.5 Sleep EEG maps the functional neuroanatomy of executive processes in adolescents born very preterm

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Submitted.

Abstract

Executive function deficits are among the most frequent sequela of very preterm birth but the underlying neuronal mechanisms are not yet fully understood. In this study, we used high-density EEG recordings during sleep to assess alterations in the functional neuroanatomy of executive processes in adolescents born very preterm. The topographical distribution of sleep slow wave activity (SWA; 1-4.5 Hz EEG power) has previously been used to map cognitive abilities and is known to reflect the intensity of the prior use of the respective neuronal networks. We assessed 38 adolescents born before 32 weeks of gestation (mean age at assessment: 12.9 (SD: 1.7), range: 10.6-16.7 years; 55.3% boys) and 43 typically-developing term-born peers (13.1 (2.0), 10.0-16.9; 48.8% boys). Executive function abilities were quantified with a composite score derived from a comprehensive task battery. All-night high-density EEG (128 electrodes) was recorded and SWA of the first hour of sleep was calculated. Executive function abilities were significantly poorer in the very preterm compared to the term-group, particularly, if the tasks demands were high ($P < .01$). The executive function composite score was positively correlated with sleep SWA in a cluster of 15 electrodes over frontal brain regions and negatively in a cluster of 14 electrodes over central brain regions after controlling for age at assessment and correcting for multiple comparisons. Within the frontal cluster, sleep SWA was higher in very preterm compared to term-born participants when controlling for executive function performance and age at assessment ($P = .02$). No difference in SWA between very preterm and term-born participants was found for the central cluster ($P = .29$). Our results demonstrate a local increase of sleep SWA over brain regions associated with executive processes in adolescents born very preterm compared to similarly performing term-born peers. Thus, sleep SWA maps the higher effort needed for executive function tasks in adolescents born very preterm.

Introduction

Very preterm birth (< 32 weeks of gestation) is a significant risk factor for neurodevelopmental deficits in childhood and adolescence (Latal, 2009). Particularly, executive functions, a set of higher-order cognitive abilities needed for goal-directed behavior (*e.g.*, inhibition, working memory, cognitive flexibility (Miyake *et al.*, 2000; Anderson, 2002)), are frequently impaired in those born very preterm (Aarnoudse *et al.*, 2012; Burnett *et al.*, 2015). We have shown recently that even very preterm children and adolescents with normal intellectual and motor abilities may be affected when the demands placed on their abilities are high (Wehrle *et al.*, 2016).

Impaired structural and functional neuroanatomy due to early disruptions of normal brain development have been suggested to underlie the neurodevelopmental deficits associated with preterm birth (Ment and Constable, 2007; Ment *et al.*, 2009; Volpe, 2009b). More specifically, impaired executive function performance was found to be related to smaller regional grey and white matter volumes (Nosarti *et al.*, 2008; Taylor *et al.*, 2011), reduced cortical thickness (Skranes *et al.*, 2012) and impaired white matter microstructure (Skranes *et al.*, 2009) in very preterm born children and adolescents. In parallel, using functional MRI, altered neural activation patterns in response to executive function tasks have been reported (Curtis *et al.*, 2006; Nosarti *et al.*, 2006; Griffiths *et al.*, 2013; Mürner-Lavanchy *et al.*, 2014). However, alterations in the functional neuroanatomy of executive processing was associated with both task-related (*e.g.*, cognitive load of the task) and subject-related (*e.g.*, individual cognitive capacity) factors and, thus, may limit the interpretation of the findings (Murphy and Garavan, 2004; Ment and Constable, 2007; Uddin *et al.*, 2010). In contrast, assessing brain activity during sleep allows the investigation of the functional anatomy of neurocognitive networks independent of factors related to wakefulness (e.g., motivation or concentration), as during sleep, individuals are virtually disconnected from the environment (Tononi and Cirelli, 2006). Importantly, high-density electroencephalography (EEG) allows the investigation of topographical patterns of brain activity by combining the superior temporal resolution of EEG recordings with high spatial resolution (Lustenberger and Huber, 2012).

The topographical distribution of sleep EEG activity has been suggested to reflect individual traits of functional neuroanatomy as it is highly stable within but varies considerably between individuals (Finelli *et al.*, 2001; De Gennaro *et al.*, 2005; Geiger *et al.*, 2012; Lustenberger *et al.*, 2016). Sleep slow wave activity (SWA; EEG spectral power between 1 and 4.5 Hz during non-rapid eye movement (NREM) sleep) may be of particular interest when investigating functional neuroanatomy as it is a marker of neural synchrony and synaptic

strength within cortical networks (Tononi and Cirelli, 2006). SWA has previously been used to map cognitive and motor skills as well as maturational aspects of functional neuroanatomy in typically-developing children and adolescents (Kurth *et al.*, 2010; Kurth *et al.*, 2012; Lustenberger *et al.*, 2016). Further, SWA topography in pediatric populations (*e.g.*, children with attention-deficit/hyperactivity disorder) was used to illustrate disease-related alterations in functional neuroanatomy compared to typical brain development (Ringli *et al.*, 2013). It is known that sleep slow waves measured in the surface EEG are a reflection of the synchronized oscillatory activity of the thalamocortical system (Steriade *et al.*, 1993; Crunelli *et al.*, 2015), and this knowledge may help the interpretation of alterations in sleep SWA following very preterm birth.

In the current study, we aimed to investigate whether the topographical distribution of SWA assessed by high-density EEG during sleep is associated with deficits in executive functions in children and adolescents born very preterm.

Materials and methods

Participants and experimental design

The eligibility criteria and the selection process for participation in the current study have been described in detail previously (Wehrle *et al.*, 2016). In short, 41 children and adolescents born very preterm (< 32 weeks of gestation) without any major neonatal brain injuries and with a normal intellectual and motor development participated in the current study. Perinatal and routine follow-up data were collected from the hospital's medical records. Those who agreed to participate did not differ from those who did not agree with regard to gestational age, birth weight, perinatal complications and intellectual and motor abilities assessed at the follow-up consultation (all $p > .05$). Participants were assessed for the current study when they were between 10 and 16 years old. For the control group, 43 typically-developing term-born peers were recruited. They were group matched to the very preterm participants in terms of sex and age at assessment. For all participants, socio-economic status (SES) was estimated using a six-point scale based on maternal education and paternal occupation (Largo *et al.*, 1989).

One week prior to the assessment, participants were instructed to keep a regular sleep-wake schedule according to their habitual bed time. Compliance was verified with self-reported sleep logs and wrist motor actigraphy (Actiwatch Plus, AW4, Cambridge Neurotechnology, Cambridge, England). Over the course of a full afternoon, cognitive abilities were assessed by an examiner who was aware of the birth status but unaware of the medical history of the

participants. After dinner, the EEG recording was prepared and participants went to bed at their habitual bed time. Wake-up times were adjusted to the participants' individual routines (e.g., school attendance). All data were collected between January and December 2013 at the Child Development Center and the Sleep Laboratory of the University Children's Hospital Zurich. The study was approved by the local ethical committee. Written informed consent was obtained from a parent as well as from participants older than 15 years. Younger participants provided oral consent. Participants were compensated with a gift certificate.

Assessment of executive functions

Detailed methods on the neurodevelopmental assessment in this cohort have been reported previously (Wehrle *et al.,* 2016). Executive functions were assessed with three subtest of the Cambridge Neuropsychological Test Automated Battery (CANTAB (2004, 2011)) and the Regensburger Wortflüssigkeits-Test (RWT (Aschenbrenner *et al.,* 2000)), a German-language verbal fluency test. The CANTAB consisted of the Stockings of Cambridge task to assess planning abilities, the Spatial Working Memory task to assess working memory and the Intra-/Extradimensional Shift task to assess cognitive flexibility. The RWT included phonetic and semantic fluency and switching subtests. The results of the subtests were averaged to reflect overall verbal fluency abilities. Each executive function task consisted of multiple trials with increasing levels of demand, for example one versus two minutes of word production in the RWT task (see Wehrle *et al.*, 2016 for details).

An executive function composite score reflecting performance in highly demanding tasks was calculated, as deficits have been shown to become clinically relevant when task demands are high (Wehrle *et al.*, 2016). For each of the four executive function tasks, the performance in low demand trials was subtracted from the performance in high demand trials to take into account individual baseline performance (i.e., performance when the demands placed on the abilities are low). Subsequently, results were z-transformed using the mean and standard deviation of the control group to obtain equal scaling of the different tasks. Z-scores were combined in a single composite score reflecting executive function abilities in highly demanding trials adjusted for baseline performance.

All-night sleep EEG recording and preprocessing

All-night EEG during sleep was recorded with a high-density EEG device with 128 channels (Electrical Geodesic Inc.; Sensor Net for long-term monitoring). The nets were adjusted to the vertex and gel electrolyte was used to fill the electrodes. Impedances were kept below 50 kΩ. EEG recordings were sampled at 500 Hz (filtered between 0.01 and 200 Hz) and referenced to the vertex (Cz). For further analyses, the data was band-pass filtered (0.5 - 50 Hz) and downsampled to 128 Hz. Sleep stages were scored for 20-second epochs according to standard criteria (Iber *et al.*, 2007) by one person who was blinded to birth status. Artifacts were identified and the corresponding epochs were removed on a 20-second basis by visual inspection and if power exceeded a threshold based on a mean power value in the 0.75-4.5 or 20-30 Hz band (Lustenberger *et al.*, 2014). Also, channels with overall bad quality were removed (on average one EEG channel per participant; range: 0 to 4). Data was re-referenced to the average of all good quality channels above the ears (in total 109 channels). Two participants born very preterm refused the EEG recording at the day of the assessment and for one participant born very preterm, data was lost due to technical difficulties. For two term-born participants, the majority of channels lost signal during the second half of the night and these participants were excluded for the analyses concerning these time points of the night. For one very preterm participant, the net was removed after 4.5 hours due to discomfort. Thus, complete datasets were available for further analyses in 38 very preterm and 43 term-born participants (37 and 41 for the analyses of later time points of the night, respectively).

Spectral analysis, power analysis and statistical analyses

Spectral analysis of consecutive 20-second epochs (fast Fourier transformation, Hanning window, average of five 4-second epochs, frequency resolution of 0.25 Hz) was performed. SWA was calculated as the mean power in the frequency band between 1 and 4.5 Hz during the first hour of NREM sleep (stage 2 and 3). This interval was selected to account for inter-individual differences in sleep episode duration and because it constitutes the most consolidated part of sleep. Additionally, SWA during the last hour of NREM sleep was calculated to investigate the stability of the effects across the night. Absolute power values were normalized for each participant by dividing the power in each electrode by the average power of all good quality electrodes above the ears. Normalized power values were used for all analyses as they have been shown previously to reliably reflect local aspects of SWA (Kurth *et al.*, 2010; Buchmann *et al.*, 2011b).

To assess the association between SWA and executive function abilities, Pearson product-moment correlation coefficients between SWA in every electrode and the executive function composite score were calculated. To correct for multiple comparisons, statistical nonparametric mapping (SnPM) using a suprathreshold cluster analysis was applied (Nichols and Holmes, 2002; Huber *et al*., 2004). *R*-values within clusters were Fisher z-transformed and the mean correlation across the electrodes within the cluster was calculated. To assess whether the association between executive function abilities and EEG power within the identified clusters is specific to the SWA frequency range, the executive function composite score was correlated to the power in every 0.25 Hz frequency bin between 1 and 25 Hz. To correct for multiple comparisons, a false discovery rate (FDR) correction was applied. Mean SWA of all electrodes within an identified cluster was calculated and compared between very preterm and term-born participants by means of univariate analyses of covariance (ANCOVA) with mean SWA within a cluster as the independent variable, birth status as a fixed factor and executive function performance and age at assessment as covariates. All analyses were performed with the software package MATLAB (MathWorks) and SPSS 22.0. The significance level was set at $p < .05$ (two-tailed).

Results

Participant characteristics, executive function abilities and sleep architecture

The mean age at assessment and the sex distribution was not significantly different between the two groups ($p > .05$, Supplementary Table 1). Major perinatal characteristics of the very preterm group are summarized in Supplementary Table 1 (for detailed perinatal data of this cohort, see (Wehrle *et al.*, 2016)).

As reported previously (Wehrle *et al.*, 2016), all participants had IQ scores in the normal range and normal motor abilities at the time of the assessment. Executive function performance was good and similar in the two groups when the task demands were low but very preterm participants scored significantly poorer than their term-born peers when the demands increased (reported previously (Wehrle *et al.*, 2016); see Fig. 1A-D). Accordingly, the overall executive function composite score was significantly lower in very preterm compared to term-born participants ($F(1, 77) = 9.374$, $p = .003$ (adjusted for age at assessment and SES), Fig. 1E).

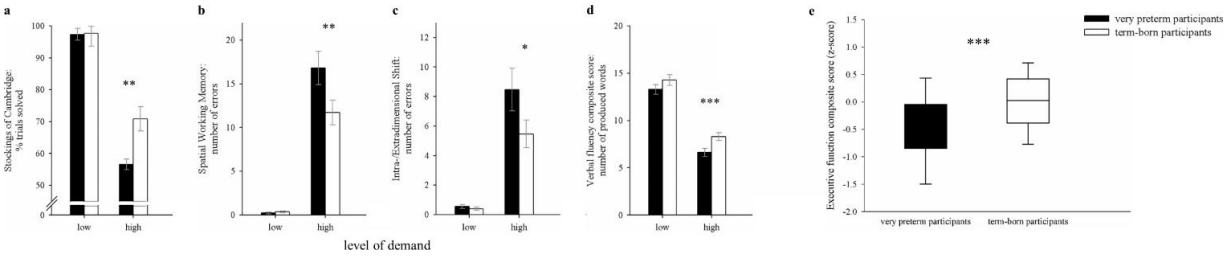

Figure 1. Executive function performance in low and high demanding trials in very preterm and term-born participants. a) Stockings of Cambridge, b) Spatial Working Memory, c) Intra-/Extradimensional Shift, d) Verbal fluency composite score. ± 1 standard error. e) Executive function composite score (performance in highly demanding trials adjusted for baseline performance). Whiskers indicate 5th and 95th percentile. *$p < .10$. **$p < .05$. ***$p < .01$, ANCOVA (adjusted for age at assessment and socio-economic status).

Sleep efficiency (*i.e.*, time asleep as a percentage of time in bed) was high at 87.0% and 87.3% in the very preterm and the term-born participants, respectively (p = .86). No significant difference between the groups was found in any of the assessed parameters of sleep architecture (Table 1). Global SWA during NREM sleep (EEG power averaged across all good-quality electrodes) was not significantly different between the groups, neither in the first nor in the last hour of the night (F(1, 78) = 2.314, p = .13 and F(1,75) = 3.144, p = .08, adjusted for age at assessment). Moreover, when directly comparing the topographical distribution of SWA, no group differences were apparent (SnPM suprathreshold cluster analysis, data not shown).

Table 1. Visually scored parameters of sleep architecture.

	Very preterm participants (n = 38)	Term-born participants (n = 43)	p[e]
Sleep efficiency[a]	87.0 (9.1; 57.7-96.4)	87.3 (8.8; 60.3-97.6)	.86
Total time in bed (in minutes)	547.1 (68.3; 271.7-649.0)	544.3 (41.0; 478.3-636.7)	.82
Total sleep time (in minutes)	477.5 (78.8; 156.7-586.7)	475.5 (60.6; 299.3-608.7)	.90
Sleep latency (in minutes)[b]	27.7 (16.7; 6.0-78.3)	24.7 (16.3; 2.3-82.7)	.46
REM sleep latency (in minutes)[c]	146.8 (59.4; 60.7-301.0)	146.3 (67.7; 58.0-301)	.97
NREM (in %[d])	76.1 (3.3; 69.3-82.6)	75.8 (5.6; 63.8-88.7)	.75
N1	11.2 (4.7; 4.3-24.9)	10.5 (3.7; 3.8-20.8)	.41
N2	50.1 (5.9; 31.3-59.7)	50.9 (5.9; 43.1-64.5)	.56
N3	14.8 (4.1; 9.5-29.6)	14.4 (4.7; 3.4-29.4)	.74
REM (in %[d])	23.9 (3.3; 17.4-30.7)	24.2 (5.6; 11.3-36.2)	.75

[a]Time asleep as percentage of total time in bed. [b]First appearance of NREM sleep (N2). [c]First appearance of REM sleep. [d] % of total sleep time. [e]independent samples *t*-test. Mean (standard deviation; range).

Regional association patterns of SWA and executive function abilities

Based on our previous knowledge about functional deficits under high demand, we expected specific, task related differences in SWA. Thus, in a first step, we identified regions where SWA was associated with executive function abilities by pooling all subjects and performing electrode-wise Pearson correlations between SWA during the first hour of NREM sleep and the executive function composite score. Fig. 2A shows the two clusters in which significant correlations between SWA and executive functions were found for the pooled group (SnPM suprathreshold cluster analysis): SWA in a cluster of 15 electrodes over frontal brain regions was positively associated with the executive function composite score. The mean correlation across the electrodes within this cluster was r = .33 (SD: .07; range: .23 to .47). In a central cluster of 14 electrodes, SWA was correlated negatively with the executive function

composite score. The mean correlation across the electrodes within this cluster was *r* = -.28 (*SD*: .04; *range*: -.35 to -.24).

To assess the stability of the topographical pattern across the night, we correlated SWA of the last hour of NREM sleep at each electrode with the executive function composite score: This analysis revealed a positive trend-level correlation in a cluster of 9 frontal electrodes showing a significant correlation ($p \leq .10$, SnPM suprathreshold cluster analysis) which overlapped with the cluster apparent during the first hour of NREM sleep (mean *r* = .27; *SD*: .04; *range*: .24 to .34). No significant cluster was identified over central brain regions.

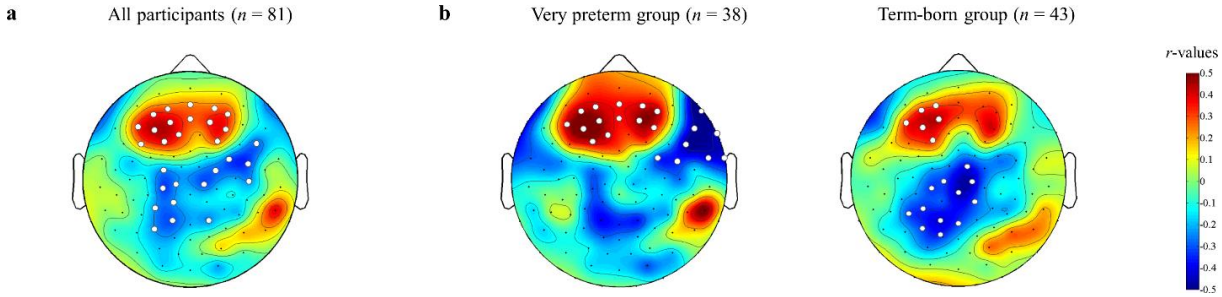

Figure 2. Association between sleep slow wave activity (SWA; EEG power between 1 and 4.5 Hz, based on the first hour of NREM sleep stages 2 and 3) and executive function abilities. Results of the electrode-wise Pearson correlations between SWA and the executive function composite score plotted on the planar projection of the hemispheric scalp model. White dots indicate clusters of electrodes showing significant correlations (defined by statistical nonparametric mapping (SnPM; suprathreshold cluster analysis to control for multiple comparison (Nichols and Holmes, 2002)). a) All participants. b) Very preterm group: frontal cluster of 13 and temporo-central of 8 electrodes showing significant correlations (mean *r* = .46; *SD*: .07; *range*: .36 to .61 and mean *r* = -.48; *SD*: .11; *range*: -.70 to -.34, respectively). Term-born group: frontal cluster of 6 and central cluster of 12 electrodes showing significant correlations (mean *r* = .38; *SD*: .05; *range*: .32 to .46; $p \leq .10$, SnPM suprathreshold cluster analysis and mean *r* = -.38; *SD*: .06; *range*: -.49 to -.32, respectively).

The association between EEG power in the frontal and central cluster and the executive function composite score was specific to the SWA frequency range. In the frontal cluster, significant correlations were found exclusively in 12 frequency bins between 1 and 4 Hz after FDR-correction. In the central cluster, EEG power was associated with executive function performance exclusively in 8 frequency bins between 1 and 3.5 Hz after FDR-correction.

We also examined whether the regional association patterns between SWA and executive function abilities were different between the two groups. The positive associations between SWA and executive function abilities over frontal brain regions was apparent in both groups but tended to be more pronounced in the very preterm group. The topographical distribution of negative associations between SWA and executive function abilities over central brain regions was more variable between the groups (Fig. 2B).

SWA difference between very preterm and term-born participants in clusters of interest

Next, we assessed whether SWA in the frontal and central clusters of interest is different between the groups when holding executive function performance constant, i.e., controlling for individual performance levels. Mean SWA of the first hour of NREM sleep in the frontal cluster was significantly higher in the very preterm group compared to the term-born group when controlling for executive function performance and age at assessment ($F(1, 77) = 5.324$, $p = .02$, Fig. 3). No significant difference between the groups was found in the central cluster ($F(1, 77) = 1.123$, $p = .29$). The results persisted when including SES as an additional covariate ($p = .05$ for the frontal and $p = .44$ for the central cluster, respectively).

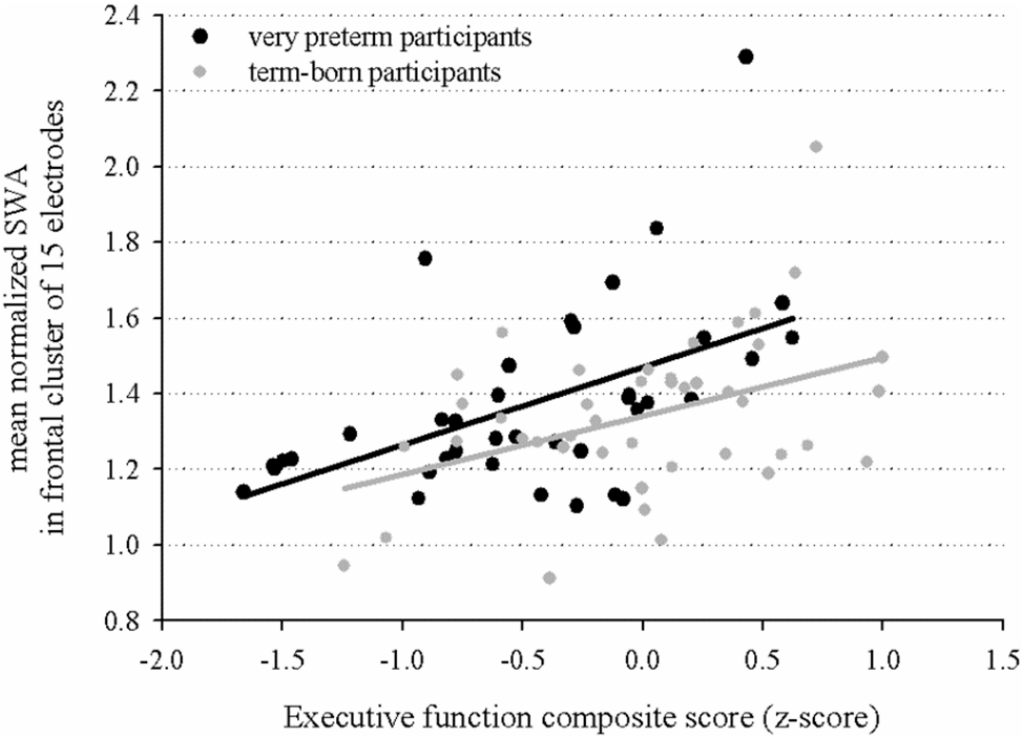

Figure 3. Association between executive function performance and mean sleep slow wave activity (normalized: absolute power in each electrode divided by average power of all good quality electrodes above the ears) in a cluster of 15 electrodes over frontal brain regions in very preterm (n = 38) and term-born (n = 43) participants. Significantly higher slow wave activity in the very preterm compared to the term-born group (F(1, 77) = 5.324, p = .02, adjusted for executive function performance and age at assessment).

To assess the stability of this group difference across the night, the analyses were repeated with mean SWA of the last hour of NREM sleep as the dependent variable. SWA was not significantly different between the groups at the end of the night after adjusting for executive function performance and age at assessment, neither in the frontal nor in the central cluster (F(1, 74) = .094, p = .76 and F(1, 74) = .002, p = .97, respectively).

Discussion

In this study, sleep SWA assessed with high-density EEG was used to map the functional neuroanatomy of executive processes in children and adolescents born very preterm in comparison to term-born peers. Positive associations between sleep SWA and executive function abilities were found in a widespread area of frontal brain regions. Within this area, individuals born very preterm exhibited more SWA compared to similarly performing term-born peers. Over central brain regions, SWA was negatively associated with executive function abilities while no difference between the groups was apparent in these regions.

The topographical distribution of the EEG power during deep sleep has been suggested to reflect individual traits of functional neuroanatomy as it is highly stable within individuals across multiple nights while it varies considerably between (Finelli *et al.*, 2001; De Gennaro *et al.*, 2005; Geiger *et al.*, 2012; Lustenberger *et al.*, 2016). Twin studies have estimated its heritability to lie above 90%, thus, making it to one of the most heritable traits of humans (De Gennaro *et al.*, 2008). Particularly, sleep SWA topography is a useful tool to map neurofunctional networks of cognitive and motor abilities in typically-developing children and adolescents (Kurth *et al.*, 2012; Pugin *et al.*, 2014; Wilhelm *et al.*, 2014). The results of the current study expand and advance these findings by demonstrating that SWA topography reliably maps the functional neuroanatomy of executive processes following very preterm birth.

The stable and frequency-specific positive association across the night between sleep SWA and executive function abilities over frontal brain regions is in line with a previous study which reported cognitive control skills to be positively related to sleep SWA over frontal brain regions in typically-developing children (Kurth *et al.*, 2012). Also, structural and functional neuroimaging studies in both typically-developing and very preterm children and adolescents found frontal brain areas to be critically involved in executive function processing (Anderson, 2002; Diamond, 2002; Nosarti *et al.*, 2008; Mürner-Lavanchy *et al.*, 2014; Ullman *et al.*, 2014; Østgård *et al.*, 2016).

Identifying potential alterations in the functional neuroanatomy of executive processes following very preterm birth was of particular interest in the current study. The association between higher SWA over frontal brain regions and better executive function abilities could be detected both in the very preterm and in the term-born group, thus, suggesting overall similar functional neuroanatomy underlying executive processes in both groups. This is in line with reports from functional MRI studies which found activation in similar anatomical regions in very preterm and term-born individuals while performing executive function tasks (Griffiths *et*

al., 2013; Daamen *et al.*, 2014). Interestingly, the cluster of significant positive associations was more widespread and pronounced in the very preterm born group compared to those born at term. It has been proposed that the recruitment of additional, less specialized brain regions may be an adaptive mechanisms employed by the brain to cope with highly demanding tasks (Just and Varma, 2007). In line with this suggestion, a functional MRI study reported increased activation in less task-specific brain regions in adults born very preterm compared to term-born peers when performing a response inhibition task (Lawrence *et al.*, 2009). Similarly, the larger cluster of electrodes with strong positive associations between SWA and executive function abilities reported here for the very preterm compared to the term-born group may reflect such adaptive mechanism of the very preterm brain.

While it is well known that frontal brain regions are crucially involved in executive processes, the association between more SWA over central regions and poorer executive function abilities, which was particularly evident in the term-born group, is more difficult to interpret. Previously, SWA in this region has been associated with the development of simple and complex motor abilities in typically-developing children (Kurth *et al.*, 2012). Interestingly, different studies have reported that reduced activity in certain brain regions while performing a task may be associated with good rather than poor performance. For example, single neuron recordings in the posterior cingulate in animals revealed that the decreased neuronal firing rate during a working memory task is associated with better performance (Hayden *et al.*, 2009). In humans, various functional MRI studies have reported that the increased suppression of the default-mode network during task execution is associated with better performance (see Anticevic *et al.*, 2012 for an overview). Accordingly, in adults born very preterm who performed comparably to term-born peers in a working-memory task, prominent suppression of the default-mode network during task performance was observed, particularly when task demands were high (Daamen *et al.*, 2014). The association between less sleep SWA over central brain regions and better executive function abilities which was observed in the current study may reflect such processes and future studies using, for example, source localization should further elaborate on this issue.

In contrast to other neuroimaging methods, EEG assessments during sleep allow the investigation of brain activity independent of subject- and task-related factors inherent to wakefulness (Tononi and Cirelli, 2006). This may be of particular relevance when investigating neurodevelopment following very preterm birth: Significant group differences in, for example, executive function performance compared to term-born peers have been reported repeatedly

(Aarnoudse *et al.*, 2012; Burnett *et al.*, 2015) and it has been suggested that performance differences between groups rather than birth status per se may alter brain activity assessed with functional MRI (Ment and Constable, 2007). The very preterm children and adolescents assessed in the current study experienced significant executive function deficits compared to their term-born peers despite normal intellectual and motor abilities. The assessment of the functional neuroanatomy of the impaired processes during sleep rather than waking allowed the unbiased identification of alterations and, consequently, may facilitate the understanding of neuronal mechanisms underlying these deficits.

The very preterm individuals assessed in this study had not suffered from any major neonatal brain injuries and we have reported previously that neither global nor regional brain volumes were significantly altered in this cohort when assessed in adolescence (Wehrle *et al.*, submitted). Also, no global alterations in the topographical distribution of sleep SWA were apparent compared to term-born peers, thus, suggesting similar general functional neuroanatomy in both groups. Importantly, using a high-density EEG recording device allowed the investigation of localized patterns of brain activity in relation to cognitive abilities. This revealed alterations in the functional neuroanatomy of executive processes, confined to frontal and temporo-central brain areas, in individuals born very preterm. Hence, in the absence of major between-group differences in neuroanatomy, the investigation of the functional integrity of specific cognitive networks may be necessary to better understand potential underlying mechanisms of deficits. Similarly, in adults born very preterm, structural brain changes could not fully explain the altered activation patterns in response to executive-type tasks in a functional MRI study (Nosarti *et al.*, 2009). Considering the aforementioned benefits of sleep EEG when investigating the functional neuroanatomy of cognitive processes, high-density recordings are particularly useful for evaluating local aspects of altered brain development following very preterm birth.

The slow waves dominating the surface EEG during deep sleep result from the synchronized oscillatory activity in the component neurons of the thalamocortical system (see Crunelli *et al.*, 2015 for an overview). As the emergence of thalamocortical connections constitutes a key neurodevelopmental process during the last trimester of pregnancy (Volpe, 2009b; Kostović and Judaš, 2010), very preterm birth, coinciding with this crucial period of brain development, puts the thalamocortical system at specific risk for adverse environmental impacts such as hypoxia/ischemia, inflammation and other neonatal complications (Volpe, 2009b). Accordingly, impaired thalamic development and disrupted thalamocortical connectivity have

been reported repeatedly in very preterm infants (Boardman *et al.*, 2006; Counsell *et al.*, 2007; Srinivasan *et al.*, 2007; Smyser *et al.*, 2010; Ball *et al.*, 2012; Ball *et al.*, 2013) and first findings suggest that such impairments of the thalamocortical system may underlie later neurodevelopmental deficits (Ball *et al.*, 2015). In the current study, SWA topography in relation to executive function abilities was different between very preterm and term-born individuals. Hence, altered thalamocortical connectivity due to early disruptions of normal brain development may underlie these differences in the functional neuroanatomy of executive processes and potentially lead to the apparent executive function deficits. Similarly, sleep SWA has previously been shown to be closely associated with the integrity of white matter tracts of frontal brain regions and the corpus callosum in typical brain development (Buchmann *et al.*, 2011a; Piantoni *et al.*, 2013). White matter injuries are common after very preterm birth and may impede subsequent brain development (Volpe, 2003, 2009b; de Kieviet *et al.*, 2012b). Importantly, diffuse white matter abnormalities have been associated with aberrant functional connectivity in executive networks in very preterm infants, thus, highlighting the close relationship between the structural and functional neuroanatomy of cognitive networks (He and Parikh, 2015). In the future, employing sleep SWA topography as a mapping tool of the functional neuroanatomy of cognitive networks in parallel to the assessment of their structural integrity may provide novel insight into how the brain copes with disruptions of typical developmental processes following very preterm birth.

Besides its potential to map the functional neuroanatomy of cognitive processes in typical and atypical brain development, sleep SWA also reflects neuroplastic processes within cortical networks: Sleep slow waves are tightly connected to the functional properties of synapses as they reflect the synchrony and synaptic strength of neuronal networks (Tononi and Cirelli, 2006). Importantly, SWA has been shown to increase as a function of the prior use of these networks during wakefulness, presumably reflecting neuroplastic processes (*i.e.*, increased synaptic strength). For example, after typically-developing children and adolescents had learned a visuo-spatial task, SWA in the subsequent night was locally increased over parietal brain regions known to be involved in visuo-spatial learning (Wilhelm *et al.*, 2014). Also, in typically-developing adolescent boys, sleep SWA was increased after three weeks of intensive working memory training over brain regions which are known to be part of the core working memory network with the increase being related to improved performance after the training (Pugin *et al.*, 2014). Sleep SWA, thus, seems to reflect both the short- and long-term use-dependent functional plasticity of cognitive networks. In the current study, a local increase of sleep SWA was observed in very preterm individuals compared to term-born peers with similar

executive function abilities exclusively over frontal brain regions, which are known to be associated with executive processing. In line with the use-dependence of SWA, this local increase of SWA may reflect plastic mechanisms of the preterm brain to cope with the high demands placed on executive function abilities. In other words, to achieve similar performance as their term-born peers, very preterm individuals may strain executive function networks more strongly, which leads to increased synchrony and synaptic strength within the relevant networks and, subsequently, to the expression of more sleep SWA during the night. Results from several functional MRI studies may support this interpretation as increased activation within relevant networks in response to different executive function tasks have been reported for children and adults born very preterm compared to term-born peers (Nosarti *et al.*, 2006; Kalpakidou *et al.*, 2014; Mürner-Lavanchy *et al.*, 2014). In parallel, childhood and adolescence have been described as developmental periods in which the demands placed on executive function abilities by the environment increase markedly (Jacobson *et al.*, 2011; Burnett *et al.*, 2013). Those born very preterm may be particularly challenged (Luciana *et al.*, 1999; Bayless and Stevenson, 2007; Wehrle *et al.*, 2016) and the deficits in a variety of executive functions which become only evident under highly demanding conditions may be a quantitative marker of these load-dependent executive function deficits following very preterm birth (Wehrle *et al.*, 2016). Compensational mechanisms of the very preterm brain, namely the stronger employment of networks, may help to cope with high demands and their failure may underlie the load-dependent deficits in executive processes exhibited by many adolescents born very preterm.

The findings of the current study suggest that not only the increased activation during task-execution but also the sleeping brain may contribute to compensational processes in executive functioning in very preterm individuals: Many studies have previously provided evidence that sleep slow waves are crucial for functional recovery processes within sensory, motor and cognitive networks and that this may underlie the continuous capacity of the brain to adapt to the environment (Van Der Werf *et al.*, 2009; Groeger *et al.*, 2014; Tononi and Cirelli, 2014; Achermann and Borbély, 2015). Accordingly, high sleep SWA in those born very preterm may facilitate functional recovery of executive function networks and, thus, support optimal performance. Future studies will need to further investigate this issue (see also Limitations).

Limitation

The cross-sectional design of the current study prohibits the assessment of developmental aspects. As childhood and adolescence is a developmental period of major structural and functional reorganization of brain networks, longitudinal assessments including several assessments of cognitive abilities and brain activity are needed to investigate developmental trajectories and the occurrence of compensational mechanisms following very preterm birth. Such data may help to gain insight into how the preterm brain copes with early disruptions of normal development and to define time windows when children may be particularly susceptible to interventions.

The participants of the current study only spent one night in the sleeping lab. This prohibited the systematic manipulation of executive function demands and the investigation of subsequent effects on sleep SWA. Instead, executive function performance under highly demanding conditions was used as a maker of load-dependent deficits and related to sleep SWA topography in a correlational manner. Future studies which record several nights of high-density EEG during sleep following performance assessments under varying executive function loads are needed to shed light on the causal role of high loads on executive function networks.

Along the same line, executive functions were only assessed in the afternoon preceding sleep and no tasks were applied in the morning. Thus, this study cannot provide information on whether sleep and sleep SWA, indeed, actively contribute to functional recovery within executive function networks and, thus, support compensational processes.

Conclusion

This is the first study to show that sleep SWA assessed with high-density EEG is a valuable tool to map the altered functional neuroanatomy of executive processes following very preterm birth. Particularly, increased SWA was apparent in very preterm individuals compared to similarly performing term-born peers over frontal brain regions, pivotal parts of the executive function network. This may reflect the increased use of this network during the day and, thus, be a compensational mechanisms of the preterm brain in response to high executive function demands. Load-dependent executive function deficits may be a consequence of inadequate functional plasticity when the demands exceed a certain level. The findings of this study add to the current knowledge on the functional neuroanatomy of executive processes following very preterm birth by investigating brain networks independent of confounding factors related to waking (e.g., attention allocation).

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Supplementary material

Supplementary Table 1. Demographic, socio-economic and perinatal data.

	Very preterm participants (*n* = 38)	Term-born participants (*n* = 43)	*p*[d]
Demographic and socio-economic data			
Age, *M* (*SD*), range (in years)	12.9 (1.7), 10.6-16.7	13.1 (2.0), 10.0 - 16.9	.56
Sex, male/female	21/17	21/22	.56
SES[a], *M* (*SD*), range	2.5 (1.0), 1-4	2.0 (0.9), 1-4	.02
Perinatal data			
Gestational age, *M* (*SD*), range (in weeks)	29.5 (2.1), 25.1-32.0	≥ 37	-
Birthweight, *M* (*SD*), range (in grams)	1277 (348), 840-1990	≥ 2500	-
Brain injuries[b]			
no brain injuries, *n* (%)	28 (73.7)	na[c]	
mild brain injuries, *n* (%)	10 (26.3)		

[a]Mean value of maternal education and paternal occupation; 1 = highest SES, 6 = lowest SES [b]brain injuries seen on neonatal ultrasound [c]parents reported no perinatal complications. [d]independent samples *t*-test.

5

Discussion

The overall aim of this thesis project was to assess executive function abilities and neuronal correlates underlying potential deficits in a group of very preterm children and adolescents who reflect the majority of today's very preterm survivors. As severe neonatal brain injuries and major neurodevelopmental impairments are rare in these individuals (Volpe, 2003; Rüegger *et al.*, 2012), diffuse alterations of the structural and functional integrity of neurocognitive networks may be of interest when investigating mechanisms underlying cognitive deficits. The current thesis project employed neuroimaging methods which have previously been used to assess the structural neuroanatomy of executive processes, namely cognitive testing and MRI. Also, the thesis contributed to a better understanding of the functional neuroanatomy of executive processes by investigating how neurophysiological characteristics of the sleep EEG are related to cognitive and mental functioning in typical brain development of healthy adults and, subsequently, transferring the findings into atypical brain development following very preterm birth. In the following chapter, the findings and potentially emerging research questions will be discussed.

5.1 Executive function abilities in today's cohorts of very preterm survivors

We identified clinically relevant executive function deficits in a group of children and adolescents born very preterm who did not experience any other neurodevelopmental impairments. Interestingly, the deficits only emerged when the demands placed on the children's abilities were high and they were particularly apparent in measures assessing abilities relevant for everyday life.

It has been suggested that performance-based (cognitive) measures of executive functioning assess underlying skills while rating-scale (behavioral) measures assess the application of those skills at home and at school (McAuley *et al.*, 2010). As environmental factors (*e.g.*, parental support, school system) may modulate the translation of skill deficits into behavioral impairments, the application of a comprehensive battery of assessment tools is

needed to fully capture the profile of executive function deficits following very preterm birth. Researchers as well as clinicians should take this into account when compiling test batteries.

The individuals who were assessed in this thesis project represent the majority of today's very preterm survivors and the finding of exclusive deficits identified for highly demanding executive processes in the absence of any other intellectual or motor problems may have important implications for future research efforts as well as clinical practice: To capture cognitive deficits and to identify the respective underlying neuronal mechanisms in the growing population of very preterm children and adolescents without any severe neurodevelopmental impairments and no major brain lesions, measurement tools with a high sensitivity across a spectrum of abilities are required. Currently, many cognitive test batteries assess abilities by employing different trials of increasing demands within a task (e.g., the Hamburg-Wechsler Intelligenztest für Kinder-IV by Petermann and Petermann, 2006), however, they do not provide age-normed reference values for individual demand levels. Rather, they use a combined score to reflect overall performance in the respective task. In the current thesis project, we showed that this approach may obscure relevant deficits: Very preterm and term-born participants performed similarly in a spatial working memory task when looking at overall performance (total revisit errors), however, significant group differences were apparent when investigating the most demanding level separately (see chapter 4.1.). Developing and applying appropriate measurement tools, thus, may crucially affect our ability to gain novel insight into how very preterm birth impacts long-term outcome.

The findings of this thesis project may also have clinical implications: As the demands placed on the abilities by the environment markedly increase at the transition to adolescence (Jacobson *et al.*, 2011; Burnett *et al.*, 2013), deficits in executive functions may only become apparent then. To ensure the timely initiation of support (e.g., counselling and therapy) and consequently minimizing the burden on the children and their families, a continuous monitoring of very preterm children beyond the transition into adolescence may be required in the future. For example, extending the routine follow-up care for very preterm infants in Switzerland beyond five years of age and including data on executive function abilities into the national registry 'SwissNeoNet' (http://www.neonet.ch/en/) may not only benefit the care of these children but also provide a valuable data base for future research efforts.

It has been reported repeatedly in typically-developing and in very preterm children and adolescents that executive function abilities are closely associated with academic achievement (Bull *et al.*, 2008; Mulder *et al.*, 2010; Best *et al.*, 2011; Rose *et al.*, 2011; Aarnoudse *et al.*, 2013). In our cohort, the parents of the very preterm individuals reported a significantly higher need for additional academic support (*e.g.*, academic coaching) than did parents of the term-born individuals ($\chi^2 = 10.262$, $p = .001$, Figure 5).

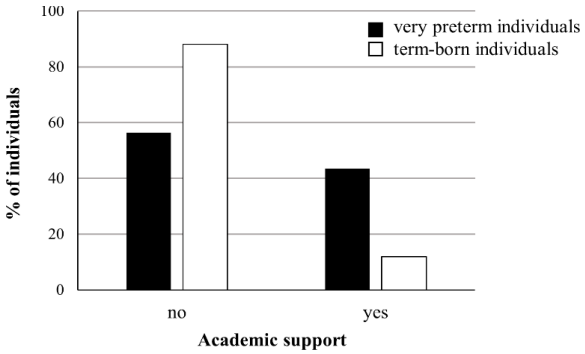

Figure 5. Percent of individuals who received one or more of the following means of academic support: Academic coaching, grade retention, additional year of kindergarten, "Einführungsklasse", any kind of special schooling.

The current study was underpowered to comprehensively investigate whether the identified executive function deficits underlie the increased need for support in the very preterm group. A detailed assessment of academic abilities with appropriate neuropsychological tests (*e.g.*, the 'Heidelberger Rechentest' to assess mathematical abilities (Haffner *et al.*, 2005)) in parallel to the assessment of executive function abilities could shed light on whether academic abilities are differentially affected by executive function deficits. Particularly, the effect of increased executive function demands on academic achievement in higher educational levels (Jacobson *et al.*, 2011) should be investigated as this may explain the widening gap in performance across elementary, middle and high school reported in very preterm individuals (Allen *et al.*, 2011). Potentially, this may result in curricular adjustments (*e.g.*, reduction of executive function demands) or the development of executive function training programs which specifically focus on the establishment of strategies needed for coping with increasing executive function demands. A study investigating the protective effect of an adaptive working memory training for academic difficulties is currently ongoing and may provide novel insight into these issues (Pascoe *et al.*, 2013).

5.2 Subcortical structures and executive function abilities

Using structural MRI and automated methods to quantify global and regional brain volume, we identified several subcortical structures which were associated with executive function abilities in children and adolescents born very preterm. More precisely, better working memory abilities were related to larger volumes in the thalamus, the hippocampus and the cerebellar white matter in very preterm individuals but not in term-born peers.

Interestingly, the associations between working memory abilities, a key executive function underlying other, more complex abilities ((e.g., planning; Miyake *et al.*, 2000), and several subcortical brain regions in the very preterm group emerged in the absence of overt brain volume differences compared to term-born peers. Similarly, associations between IQ and the integrity of white matter microstructure have been reported in very preterm but not in term-born adolescents in the absence of group differences in white matter development (Feldman *et al.*, 2012). It was suggested that structural neuroimaging may contribute to understanding individual differences after preterm birth but may not be feasible to differentiate a relatively high-functioning group of preterm children from typically-developing term-born peers (Feldman *et al.*, 2012). Investigating neuroanatomical correlates of executive function deficits within a relatively homogenous group of children and adolescents born very preterm with normal intellectual and motor abilities may, thus, be a promising approach to study the structural integrity of neurocognitive networks and, consequently, to better understand neuronal mechanisms underlying specific deficits. Accordingly, the findings of the current thesis project emerged from such a within-group approach and did, indeed, reveal important insight into neuroanatomical correlates of executive processes, namely highlighting the specific relevance of subcortical regions, particularly the thalamus, in the very preterm group.

The assessment of microstructural changes in tissue integrity using quantitative MR relaxometry (DESPOT1-HIFI method; Deoni, 2007) in the same cohort of very preterm and term-born individuals further points towards a specific vulnerability of subcortical structures, particularly the thalamus, in the very preterm brain: After correcting for age at assessment and sex, T1 relaxation times were significantly longer in the thalamus and the caudate and shorter in the insula in the very preterm compared to the term-born group ($p < .05$, FWE corrected; Figure 6, unpublished results provided by R. Tuura O'Gorman).

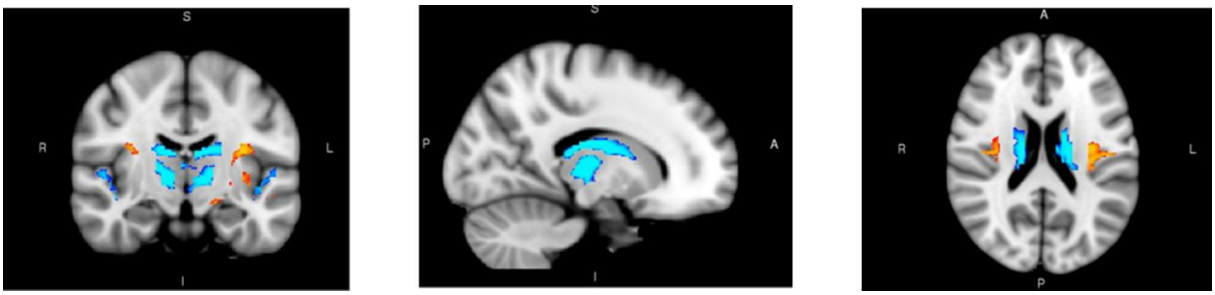

Figure 6. Coronal, sagittal and axial view of the brain. T1 relaxation times (assessed with DESPOT1-HIFI method) were increased in very preterm individuals in the thalamus and caudate (blue) and decreased in the insula (blue). $p < .05$, FWE correction controlling for age and sex.

This shows that microstructural tissue integrity may be altered in the very preterm brain even in the absence of overt volumetric differences and that subcortical structures may be at particular risk for impairments. The functional relevance of these alterations has yet to be determined, however, preliminary results suggest that they may be related to long-term outcome: While no significant association was apparent between subcortical tissue integrity and IQ or any of the executive function measures available for this cohort (all $p > .05$), longer T1 relaxation time, particularly in the caudate, was related to the number of therapies (e.g., speech therapy, learning therapy, physiotherapy) the very preterm children had received up to the time of the assessment (*e.g.*, for left caudate: Spearman's rho = .384, $p = .04$). This measure presumably reflects general outcome rather than specific neurodevelopmental deficits and may, thus, be of interest when monitoring development in children and adolescents born very preterm. Interestingly, in very preterm adolescents, altered development of the caudate has previously been associated with signs of attention-deficit/hyperactivity disorder (ADHD) assessed by parental ratings on a questionnaire while no associations with any performance-based measures of neurodevelopmental outcome were reported (Nosarti *et al.*, 2005). ADHD is a neurodevelopmental disorder strongly linked to executive function deficits (Willcutt *et al.*, 2005), thus, it is possible that executive function abilities mediate the relation between caudate development and behavioral outcome.

Considering the coincidence of very preterm birth with important neurogenetic events related to the development of subcortical regions, their specific vulnerability may not be surprising: It has been shown repeatedly that adverse impact factors associated with premature exposure to the extrauterine environment affect both the subplate neurons and the waiting afferents in the transient subplate zone (Kostović and Jovanov-Milošević, 2006; Volpe, 2009b; Kostović and Judaš, 2010). Both are closely associated with the development of subcortical structures (Chambers *et al.*, 1990; Volpe, 2009b) and preterm birth, thus, puts their integrity at significant risk. The findings of the current thesis project suggest that subcortical structures may

constitute specific neuronal correlates of subsequent executive function deficits, presumably as microstructural tissue integrity is altered even in the absence of overt volume reductions. Consequently, subcortical regions, particularly of the thalamus, should be in the focus when developing novel neuroprotective therapies.

5.2.1 Interventions to protect structural correlates of executive function abilities?

The results of the current thesis project suggest a pivotal role of the structural integrity of subcortical regions, particularly the thalamus and the hippocampus, for efficient executive processing. This raises the question whether subcortical structures may be protected against adverse impact factors associated with very preterm birth.

A recent randomized-controlled trial investigated the neuroprotective effect of early (within the first 42 hours of life) administration of recombinant human erythropoietin (rhEPO) on brain development in infants born very preterm (clinical trial no. NCT00413946). Reduced rates of grey and white matter abnormalities (Leuchter *et al*., 2014) and improved white matter microstructure (O'Gorman *et al.*, 2014), particularly of the corpus callosum and the internal capsule, in infants who received rhEPO compared to those who received placebo on structural MRI at term-equivalent age were reported. Early alterations of both grey and white matter development have been shown to crucially impact subsequent brain development, particularly of subcortical regions: For example, diffuse white matter abnormalities were found to be associated with deep grey matter growth failure, particularly of the thalamus (Boardman *et al.*, 2006; Srinivasan *et al*., 2007). It may, thus, be hypothesized that early rhEPO administration beneficially affects the development of subcortical regions in individuals born very preterm, and consequently may improve executive function abilities.

General motor and cognitive abilities at 24 months of age in the above-mentioned study were not significantly different between children who had received rhEPO and those who had received placebo (Natalucci *et al.*, 2016). Complex cognitive processes, particularly executive functions, only start to develop as children get older (Anderson, 2002), potentially preventing the identification of beneficial effects of neuroprotective interventions for these abilities at an early age. Accordingly, another study investigating the effect of early EPO administration on neurodevelopmental outcome reported better executive function abilities at age four in very preterm children with scores approaching those of term-born peers, however, the neuronal mechanisms underlying these effects were not explored (Ohls *et al.*, 2016). In the future, the long-term effects of early EPO administration on both brain structure, particularly subcortical regions, and executive function abilities in school-age children and adolescents need to be

assessed to determine the clinical relevance of this neuroprotective agent. Currently, a project exploring these issues is planned at the Department for Neonatology, University Hospital Zurich.

5.3 Functional integrity of brain networks in typical and atypical brain development

We assessed the functional integrity of brain networks and its relation to cognitive and mental functioning using high-density EEG recordings during sleep: We started by showing in healthy adults that sleep spindles, a key characteristic of NREM sleep, are related to cognitive and mental functioning and reflect the integrity of the thalamocortical system. Next, we applied these findings in our clinical population of children and adolescents born very preterm.

5.3.1 Sleep spindles as a marker of thalamocortical integrity and efficiency

Sleep spindles have been suggested to reflect the integrity and the efficiency of the thalamocortical system as they are generated by the thalamic reticular nucleus and need the integrity of both thalamocortical and corticothalamic connections for their full expression and synchronization (De Gennaro and Ferrara, 2003; Steriade, 2003).

First, we investigated whether the association between sleep spindle density and symptom severity reported in schizophrenic patients extends to the frequent schizophrenia-like experiences in the healthy population. This could further establish the utility of sleep spindles as a marker of the integrity and efficiency of the thalamocortical system. We found significant negative associations between sleep spindle density and a person's proneness to schizophrenia-like experience and thoughts. Also, lower thalamic glutamine and glutamate levels, another neurobiological marker of schizophrenia, were significantly related to sleep spindle density. These findings not only support the theory of a continuum between normal mental functioning and the psychosis found in schizophrenic patients but also provides further evidence that sleep spindles reliably assesses the integrity and efficiency of the thalamocortical system.

Next, we focused on how different sleep spindle characteristics are related to declarative memory performance in healthy adults. Previous results had been mixed with differences between findings presumably driven by the heterogeneous spindle measures which were applied. Assessing multiple nights allowed for the investigation of both trait-like (average across nights) and state-like (difference between nights) aspects of sleep spindles in relation to memory performance. We found trait-like aspects of slow spindles density (number of sleep spindles/minute) to be positively and trait-like aspects of fast spindles density to be negatively associated with memory performance, *i.e.*, declarative memory consolidation assessed as

overnight retention. These associations were apparent on a global level. In contrast, state-like aspects of integrated spindle activity were positively related to differences in overnight retention in specific brain regions. Our findings highlight the importance of the sensible choice of spindle measures and the adoption of a multidimensional approach when investigating associations between sleep spindles and cognitive abilities. Particularly when investigating atypical brain development, this may be crucial to understand how impairments of the functional integrity of the thalamocortical system may be related to cognitive abilities.

Very preterm birth has been shown to specifically impair thalamocortical development (Kostović and Jovanov-Milošević, 2006; Counsell *et al.*, 2007; Kostović and Judaš, 2010; Smyser *et al.*, 2010; Ball *et al.*, 2012; Ball *et al.*, 2013). Thus, assessing the integrity of the thalamocortical system using high-density sleep EEG may be a promising approach to gain novel insight into functional alterations of thalamocortical connections and potential consequences for cognitive and mental outcome. To do so, we first investigated alterations in the topographical distribution of spindle activity (quantified as EEG power between 12 and 15 Hz) in very preterm children and adolescents compared to term-born peers. Overall, age-appropriate patterns of distribution (Kurth *et al.*, 2010) were found for both groups. However, we identified 23 electrodes located over centro-parietal brain regions where spindle activity was significantly lower (mean reduction: 10.9 [±2.4] %) and a smaller cluster of five electrodes over frontal brain regions where spindle activity was higher (mean increase: 14.3 [0.7] %) in the very preterm than in the term-born group (Figure 7a and 7b). These alterations presumably reflect impairments of the thalamocortical integrity in the very preterm brain and potential consequences for neurodevelopmental outcome are of interest.

A study recording high-density EEG during sleep in typically-developing children has reported significant associations between spindle activity and IQ over central and parietal brain regions (Geiger *et al.*, 2012). Building on these findings, we investigated whether spindle activity is related to IQ in our cohort of typically-developing and very preterm children and adolescents. We found significant associations between more spindle activity and higher IQ over centro-parietal brain regions (Figure 7c). Potentially, the reduced centro-parietal spindle activity in very preterm children and adolescents reflects poorer efficiency of the thalamocortical system (Fogel and Smith, 2011) and, thus, may underlie the lower (despite being in the normal range) IQ in the very preterm group.

The increased spindle activity in very preterm individuals relative to term-born peers over frontal brain regions seems counterintuitive at first but may be explained in light of

findings of a non-linear relationship between sleep spindles and cognitive performance with both low and high levels of spindles potentially being maladaptive (Fogel *et al.*, 2010). Further analysis focusing on specific spindle characteristics, for example spindle density and duration, could shed further light on how local alterations in sleep spindles are related to cognitive outcome, particularly executive functioning, following very preterm birth.

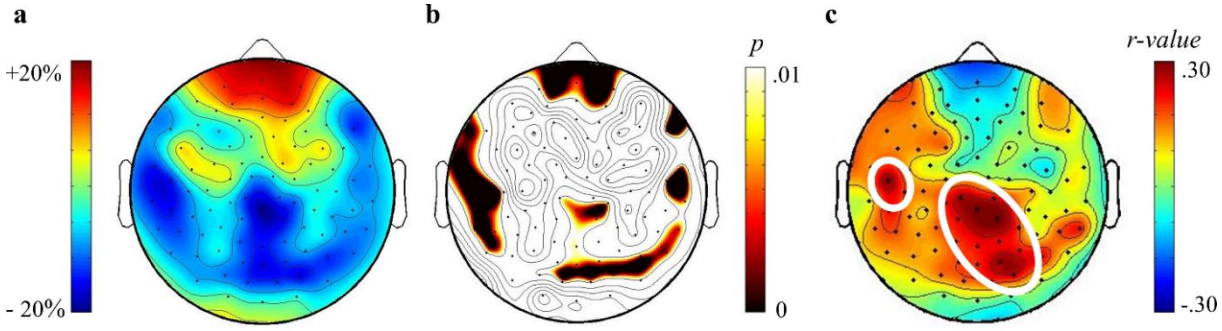

Figure 7. a) Increased and decreased sleep spindle activity (EEG power between 12 and 15 Hz) in the very preterm compared to term-born group. b) Significant *p*-values of electrode-wise unpaired Student's *t*-test to compare spindle activity between individuals born very preterm and at term. c) Electrode-wise Pearson's correlation between spindle activity and IQ. White circles indicate clusters of significant electrodes.

Interestingly, a large population-based cohort study in Sweden has recently reported that very preterm born young adults are at a 2.5-fold risk to be hospitalized due to a psychotic disorder compared to the average population (Nosarti *et al.*, 2012). As schizophrenia and other psychotic disorders have been related to impaired thalamocortical communication (Vukadinovic, 2011) with reduced sleep spindles being a hallmark of the disease (Ferrarelli *et al.*, 2007; Ferrarelli *et al.*, 2010), it would be interesting to investigate whether altered sleep spindle characteristics in very preterm individuals are related to the risk of developing psychotic disorders. As executive impairments have been found to be the most enduring and difficult to treat in schizophrenic patients (Eisenberg and Berman, 2010), a common underlying pathway of executive function deficits and the risk for psychotic disorders via impairments of the thalamocortical system due to very preterm birth could be hypothesized. In the current thesis project, we did not assess any symptoms of psychiatric disorders or psychosis-like experiences and, thus, cannot further contribute to this questions and future studies should aim to do so.

5.3.2 Sleep SWA to map the functional neuroanatomy of executive processes

Sleep SWA is the second important characteristic of NREM sleep and reflects the synchronized oscillatory activity of the thalamocortical system (Crunelli *et al.*, 2015). Its topographical distribution across the scalp has previously been shown to reflect individual traits of neuroanatomy (Finelli *et al.*, 2001; De Gennaro *et al.*, 2005; Geiger *et al.*, 2012; Lustenberger

et al., 2016) and has been used to map motor and cognitive abilities in typically-developing children (Kurth *et al.*, 2012; Pugin *et al.*, 2014; Wilhelm *et al.*, 2014). Accordingly, using SWA topography, we were able to map a network of frontal and central brain regions related to executive function abilities in children and adolescents born very preterm and at term. Both positive and negative associations (over frontal and central brain regions, respectively) between SWA and performance were apparent, presumably reflecting the complex functional neuroanatomy underlying efficient processing. Using structural and functional neuroimaging and neurophysiological methods, similar brain regions have previously been associated with executive function abilities (Anderson, 2002; Diamond, 2002; Hayden *et al.*, 2009; Anticevic *et al.*, 2012; Ullman *et al.*, 2014). Consequently, sleep SWA appears to be a feasible tool to investigate neuronal correlates of executive functions independent of factors related to waking, for example attention allocation or task-performance.

Besides its potential to map the functional neuroanatomy of cognitive processes, sleep SWA has also been suggested to be crucially linked to neuronal plasticity: During wakefulness, when interacting with the environment, synaptic strength increases. This has, for example, been demonstrated by a continuous increase of cortical excitability over the course of a day as measured by transcranial magnetic stimulation (Huber *et al.*, 2013). Due to limited energy supply and space, synaptic strength cannot be increased infinitely and is required to be reduced periodically to ensure the continuous capacity for neuroplastic adaptation. The synaptic homeostasis hypothesis (SHY) suggests that this renormalization of synaptic strength occurs during slow wave sleep (Tononi and Cirelli, 2006, 2014). Accordingly, cortical excitability returns to baseline level after a night of sleep (Huber *et al.*, 2013). Similarly, SWA declines across NREM cycles over the course of a night to reach baseline levels in the morning (Achermann and Borbély, 2015). Also, SWA has been shown to locally increase in response to the intense prior use of specific networks (Huber *et al.*, 2004; Pugin *et al.*, 2014; Wilhelm *et al.*, 2014), thus, presumably reflecting increased synaptic strength within these networks. Sleep SWA, thus, seems to be a valuable tool to assess plastic processes of the brain.

In this thesis, investigating neuroplastic processes as reflected by sleep SWA within the identified executive function network revealed important insight into how the very preterm brain copes with demands: Sleep SWA within the frontal cluster of electrodes (showing a positive association with executive function abilities), was significantly higher in very preterm children and adolescents compared to similarly performing term-born peers. This may reflect use-dependent neuroplastic adaption processes apparent in the very preterm brain. To achieve similar performance as their term-born peers, very preterm children and adolescents may need

to strain the respective neuronal networks more intensely which, then, is reflected by an increase of sleep SWA during the night over the respective brain regions due to the higher intensity of prior network use. Interestingly, in healthy adults, it has been shown that sleep SWA in relevant brain regions was higher during a nap following a difficult but not an easy learning task (Schmidt *et al.*, 2006). This may further suggest sleep SWA as an electrophysiological marker of load-dependent neuroplastic processes in involved networks. Longitudinal studies should investigate whether these presumed coping mechanisms employed by the very preterm brain remain functional and successful across development or whether the increased strain, which is placed on the neuronal networks by high executive function demands, becomes detrimental to performance at some point and, consequently hinders optimal development.

Many studies have previously provided evidence that sleep slow waves are crucial for functional recovery processes within sensory, motor and cognitive networks and that this may underlie the continuous capacity of the brain to adapt to the environment (Van Der Werf *et al.*, 2009; Groeger *et al.*, 2014; Tononi and Cirelli, 2014; Achermann and Borbély, 2015). Also, studies employing learning paradigms have provided evidence for the active involvement of sleep SWA in neuroplastic process as the learning-induced local increases in sleep SWA over relevant brain areas were found to correlate with improved performance after sleep (Huber *et al.*, 2004; Wilhelm *et al.*, 2013; Wilhelm *et al.*, 2014). An adequate amount of sleep may, thus, be particularly important for children and adolescents born very preterm to allow for the functional recovery of neuronal networks and sleep-related neuroplastic processes and consequently, efficient executive functioning during the day. Also, it has been shown that sleep SWA may be enhanced by external stimulation (Ngo *et al.*, 2013a) and that SWA enhancement may benefit cognitive processes the following day (Ngo *et al.*, 2013b). Consequently, future studies should investigate whether boosting sleep SWA in children and adolescents born very preterm could improve cognitive performance, particularly executive functioning, and whether this may help to reduce deficits following very preterm birth.

In healthy adults, it has been shown that sleep slow waves are closely related to the microstructural integrity of white matter, particularly of frontal interconnected networks (Piantoni *et al.*, 2013). Also, the volume of the corpus callosum, particularly of the anterior segment which connects prefrontal brain regions (Hofer and Frahm, 2006), has been found to be associated with sleep SWA (Buchmann *et al.*, 2011a). It has, thus, been suggested that the integrity of white matter fiber tracts, particularly of the corpus callosum and those connecting frontal to other brain regions, are crucial for the synchronization of sleep slow waves across widespread cortical areas (Buchmann *et al.*, 2011a; Piantoni *et al.*, 2013). In very preterm

children and adolescents, impaired corpus callosum development has been reported repeatedly (Nosarti *et al.*, 2004; Caldú *et al.*, 2006; Allin *et al.*, 2007; Narberhaus *et al.*, 2007; Narberhaus *et al.*, 2008) with impairments of the anterior part of the corpus callosum being specifically associated with poor executive function abilities (Narberhaus *et al.*, 2008). It should be investigated how white matter microstructure integrity, particularly of the frontal lobe and the corpus callosum, is associated with the altered functional neuroanatomy of executive processes which was reflected by altered sleep SWA in this thesis project. This may provide novel insight into how the structural and functional integrity of executive networks are related and how this may underlie deficits and compensational mechanisms following very preterm birth. Together with the fact that the thalamocortical network has been described as 'a single slow-wave-generating unit (Crunelli *et al.*, 2015)', this suggests SWA and SWA topography as a valuable tool when investigating neuronal mechanisms underlying cognitive processes in those born very preterm

Together, the results of this thesis project further establish the utility of high-density EEG recorded during sleep to investigate the functional integrity of neuronal networks, particularly involving the thalamocortical system, which underlie cognitive and mental abilities. Importantly, while this applies to typical brain development, our results suggest that sleep EEG may also provide valuable insight into atypical brain development, namely following very preterm birth.

5.4 How are structure and function related in typical and atypical brain development?

A question that arises when investigating the integrity of neuronal networks is how structure and function are related. Honey and colleagues (2010) have comprehensively approached this question by reviewing data from both animal and human studies but also large-scale computational models. They suggest that strong structural connections between regions within neuronal networks likely go along with strong functional connectivity. In contrast, strong structural connectivity cannot be inferred on the basis of strong functional connectivity as functional links may exist via indirect structural connections (Honey *et al.*, 2010). This may, consequently, suggest the need for the parallel assessment of both the structural and functional integrity of neuronal networks to understand both normal and impaired processing.

Also, it has been reported that structural plasticity may be driven by functional plasticity: For example, an intensive working memory training in healthy adults resulted in improved white matter integrity in tracts within brain networks activated by the training (Olesen

et al., 2004; Takeuchi *et al.*, 2010). Presumably, myelination of the fiber tracts was changed due to the increased glutamate release onto oligodendrocytes along the axon during action potentials (Piantoni *et al.*, 2013). With regard to sleep, it has been suggested that prolonged and repeated high level of oscillatory activity of the thalamocortical system may conceivably strengthen an individual's white matter integrity (Piantoni *et al.*, 2013). Considering the increased SWA in brain regions associated with executive function processes in very preterm children and adolescents, this may be of particular interest as the increased activity may improve the structural integrity of these networks.

Besides considering how structural and functional integrity may interact within the same network, also potential functional adaptation in compensatory networks in response to structural impairments in primary networks may be of interest, particularly when investigating atypical brain development. Recently, a study in very preterm born adults who had suffered perinatal brain injuries (PBI) provided the first evidence of compensatory functional plasticity of the working memory network in response to early structural insult: Individuals born very preterm who had suffered from PBI had significantly reduced brain volumes in the dorsal cingulum (a key fiber tract connecting frontal and parietal working memory structures) compared to individuals born very preterm without signs of PBI. In parallel, the individuals born very preterm with PBI showed a relative overactivation of the perisylvian cortex, a non-task-specific brain area. Importantly, the cingulate volume reduction was significantly correlated with the perisylvian overactivation, which, in turn, was related with better performance, particularly as the task demands increased (Froudist-Walsh *et al.*, 2015). This provides evidence that functional adaptation within the very preterm brain may be crucial to overcome structural impairments and that the assessment of both structure and function are needed to fully understand how the very preterm brain copes with demands by the environment. Future studies should investigate how structure and function of the thalamocortical system are related to allow for optimal executive processing in those born very preterm.

5.5 How can the current findings improve long-term outcome after very preterm birth?

As the underlying neuronal mechanisms of efficient executive functioning and the respective deficits and potential compensatory mechanisms of the very preterm brain become clearer, interventional approaches taking into account this knowledge should be developed. In typically-developing children and adults, it has been repeatedly demonstrated that executive function training impacts the structural and functional integrity of the underlying networks and that these changes result in improved performance after training (Olesen *et al.*, 2004; Takeuchi

et al., 2010; Jolles *et al.*, 2013; Takeuchi *et al.*, 2013; Pugin *et al.*, 2014). In very preterm children and adolescents, computerized working memory training has been shown to lead to improvements in working memory abilities (Løhaugen *et al.*, 2011; Grunewaldt *et al.*, 2013; Lee *et al.*, 2016), however, to date, the respective neuroplastic processes are unclear. It should be investigated whether similar training-induced changes as reported in typical brain development underlie performance improvements in very preterm individuals or whether the engagement of compensatory networks benefits performance. This may then influence the development of novel training programs.

Additionally, future interventional approaches could exploit the fact that plastic processes in the brain occur in an experience-dependent manner in a more comprehensive way: The environment a child grows up in provides a great variety of experiences, all of which shape the developing brain. Family socio-economic status and related factors such as different parenting behaviours have repeatedly been shown to impact brain morphology and functioning in typically-developing individuals (Kishiyama *et al.*, 2009; Jednoróg *et al.*, 2012; Noble *et al.*, 2012; Whittle *et al.*, 2014; Noble *et al.*, 2015). Frontal and subcortical brain regions, mainly hippocampal structures, were found to be particularly impacted by family factors (Rao *et al.*, 2010; Hanson *et al.*, 2011; Luby *et al.*, 2012; Staff *et al.*, 2012; Lawson *et al.*, 2013; Tomalski *et al.*, 2013). The findings reported in this thesis have illustrated that alterations in frontal and subcortical regions may underlie executive function deficits in children and adolescents born very preterm. Accordingly, developing interventional approaches which target modifiable factors of the family environment (e.g., trainings to enhance parenting capacity (Colditz et al., 2015)) may be a feasible way to strengthen the structural and functional integrity of brain regions involved in executive functioning and, consequently, may benefit executive function abilities of children and adolescents born very preterm.

5.6 Limitations

While the multimodal approach that we applied in this thesis project provided interesting insight into the structural and functional neuroanatomy of executive processes following very preterm birth, some limitations should be addressed.

The sample size of the study was relatively small and some group differences did not reach statistical significance, presumably due to limited power (*e.g.*, lack of brain volume differences). Considering the demanding protocol for the study participants and their families, it may, however, be difficult to increase the study sample considerably. In turn, a more limited

protocol, for example including a nap instead of an overnight sleep recording, could be helpful in the attempt to recruit larger numbers of study participants.

The cross-sectional design of this study prohibited the investigation of causal relationships and longitudinal changes in executive function abilities and underlying neuronal mechanisms. Considering the age of our study cohort (ranging from 10.0 to 16.9 years), this would, however, have been of great interest as both executive functions and respective neuronal correlates undergo major changes during this time (Anderson, 2002). Accordingly, to understand how executive function deficits and underlying neuronal mechanisms evolve over time, longitudinal assessments are crucial and should be the focus of future studies. However, again considering the demanding study protocol, it may be difficult to implement repeated measurements over time.

In the healthy adults, we were able to repeatedly measure both cognitive performance (i.e., declarative memory) and brain functioning (i.e., sleep EEG). This allowed us to investigate both state- and trait like aspects, thus, providing important information about changes over time. However, this approach has other disadvantages, as it is for example, difficult to generalize the results of healthy young male students to the general public.

The structural neuroimaging and electrophysiological measures that we used in this study cannot shed light on how the brain functions while performing tasks. The combination of our task-independent tools to assess the structural and functional neuroanatomy of executive processes with other methods, for example, functional MRI or EEG measures of evoked responses during task performance, could help our understanding of how the very preterm brain executes tasks.

5.7 Concluding remarks

In today's cohorts of very preterm survivors, brain development is characterized by diffuse alterations of subcortical regions, particularly the thalamus, cortical networks and the respective connecting pathways. Consequently, the structural and functional integrity of the thalamocortical system as a whole should be considered when investigating neuronal mechanisms of higher-order cognitive deficits in this population. By approaching this issue with a multimodal design, this thesis project has added a piece to this complex puzzle and future studies taking into account the current findings may not only advance our scientific understanding but may also help to further improve the long-term outcome after very preterm birth and, thus, support these children and their families in overcoming the struggles of an early start to life.

6

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Curriculum Vitae

Name	Flavia Maria Wehrle
Date of Birth	July 8th 1986 in Winterthur, Switzerland
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Education

03/2012 – present	Ph.D. student at the University of Zurich, Faculty of Arts and the Ph.D. programs at the Department of Psychology (Ph.D. in Psychology) and at the 'Integrative Molecular Medicine (imMed)' of the Zurich Center for Integrative Human Physiology (ZIHP)
10/2005 – 03/2011	Master of Science (MSc)/lic. phil. in Psychology at the University of Zurich
07/2003 – 06/2004	Foreign Exchange Year at Gibbon Fairfax Winthrop (GFW) High School, Winthrop, MN, USA
08/1999 – 07/2005	Kantonsschule Rychenberg (High School), Winterthur

Professional experience

03/2012 – present	University Hospital Zurich, Department of Neonatology and University Children's Hospital Zurich, Child Development Center (Research assistant)
09/2011 – 02/2012	University/ETH Zurich, Division Public & Organizational Health (Research assistant)
07/2010 – 09/2010	University of Zurich, Chair for Cognitive Social Psychology (Semester assistant)

Grants and Awards

06/2016 **SwissPedNet Translational & Clinical Research Award**

'EEG Sleep Slow Wave Activity as a Potential Marker of Load-Dependent Executive Function Deficits in Very Preterm Children'

SwissPedNet Translational & Clinical Research Session at the Annual meeting of the Swiss Society of Pediatrics, Bern, Switzerland

http://www.swisspednet.ch/research-session/award-2016/

10/2015 **Award for the Best Oral Presentation**

'EEG Sleep Slow Wave Activity as a Potential Marker of Load-Dependent Executive Function Deficits in Very Preterm Children'

Annual meeting of the Children's Research Center, University Children's Hospital Zurich

08/2015 **ZIHP Sprint Fellowship Grant** (CHF 30'073.-)

'Does Thalamocortical Connectivity Predict Executive Functioning in Children and Adolescents Born Very Preterm?'

Zurich Center for Integrative Human Physiology (ZIHP)

http://www.zihp.uzh.ch/en/research/sprintfellowships.html

06/2015 **Award for the Best Poster Presentation**

'Altered Topography of Sleep Spindle Activity in Very Preterm Born Children and Adolescents'

Annual meeting of the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SSSSC), Interlaken, Switzerland

03/2015 **Nominee for 'Mercator Award for Junior Researcher 2015'**

University of Zurich

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Publication list

Peer-reviewed journal articles

2016 **Wehrle, F.**, Kaufmann, L., Benz, L., Huber, R., O'Gorman R., Latal, B., & Hagmann, C. *Very Preterm Adolescents Show Impaired Performance With Increasing Demands In Executive Function Tasks.* Early Human Development. 2016; 92: 37-43.

2015 Lustenberger, C., **Wehrle, F.**, Tüshaus, L., Achermann, P., & Huber, R. *The Multidimensional Aspects of Sleep Spindles and Their Relationship to Word-Pair Memory Consolidation.* Sleep. 2015; 38: 1093-1103A.

Lustenberger, C., Murbach, M., Tüshaus, L., **Wehrle, F.**, Kuster, N., Achermann, P. & Huber, R. *Inter-individual Variation and Intra-individual Stability of the Effects of Pulsed RF EMF Exposure on the Human Sleep EEG.* Bio Electro Magnetics. 2015; 36: 169-177.

2014 Lustenberger, C., O'Gorman, R., Tüshaus, L., **Wehrle, F.**, Achermann, P. & Huber R. *Sleep Spindles Predict Schizotypal Personality Traits and Thalamic Glutamine/Glutamate in Healthy Subjects.* Schizophrenia Bulletin. 2014; 41: 522-531.

submitted **Wehrle, F.**, Buchmann, A., Guggenberger, R., Huber, R., Latal, B., O'Gorman, R. & Hagmann, C. *Subcortical Cerebral Volume Is Associated With Working Memory Performance in Adolescents Born Very Preterm.*

submitted **Wehrle, F.**, Latal, B., O'Gorman, R., Hagmann, C. & Huber, R. *Sleep EEG Maps the Functional Neuroanatomy of Executive Processes in Adolescents Born Very Preterm.*

in preparation **Wehrle, F.**, Wood, T., Buchmann, A., Latal, B., Huber, R., Deoni, S., Barker, G., O'Gorman, R. & Hagmann, C. *Quantitative T1 Relaxation Times in Very Preterm Children and Adolescents.*

in preparation **Wehrle, F.**, Latal, B., O'Gorman, R., Hagmann, C. & Huber, R. *Altered Sleep Spindle Activity May Reflect Impaired Thalamocortical Connectivity in Very Preterm Children and Adolescents.*

Oral presentations

2016 **F. Wehrle**, B. Latal, R. O'Gorman, C. Hagmann & R. Huber (June 9th - 10th). *EEG sleep slow wave activity as a potential marker of load-dependent executive function deficits in very preterm children* (Annual Meeting of the Swiss Society of Pediatrics, Bern, Switzerland)

2015 **F. Wehrle,** B. Latal, R. O'Gorman, C. Hagmann & R. Huber (October 29th). *EEG Sleep Slow Wave Activity as a Potential Marker of Load-Dependent Executive Function Deficits in Very Preterm Children (*Annual meeting of the Children's Research Center, University Children's Hospital Zurich)

F. Wehrle, B. Latal, R. O'Gorman, C. Hagmann & R. Huber (September 15th - 20th 2015). *Altered Sleep Spindle Activity May Reflect Impaired Thalamocortical Connectivity in Very Preterm Children and Adolescents* (1st Congress of Joint European Neonatal Societies, Budapest, Hungary)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (June 18th 2015). *Executive functions in very preterm children and adolescents impair with increasing task demands* (Follow-up Group meeting, Swiss Neonatal Network & Follow-Up Group, Bern, Switzerland)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, C. Hagmann, & R. Huber (April 25th - 28th 2015): *Reduced Sleep Spindle Activity May Reflect Impaired Thalamocortical Connectivity in Very Preterm Children and Adolescents* (Pediatric Academic Societies (PAS) Annual Meeting, San Diego)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, C. Hagmann, & R. Huber (April 9th, 2015): *Reduced Sleep Spindle Activity May Reflect Impaired Thalamocortical Connectivity in Very Preterm Children and Adolescents* (14th Day of Clinical Research, University Hospital Zurich)

2014 **F. Wehrle**, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (October 17th - 21st 2014): *Impaired Executive Functions in Complex Tasks in Children and Adolescents Born Very Preterm* (5th Congress of the European Academy of Paediatric Societies, Barcelona, Spain)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, C. Hagmann, & R. Huber (September 16th - 20th 2014): *Working Memory Ability and Topographical Distribution*

of Sleep Slow Wave Activity in Children and Adolescents (22nd Congress of the European Sleep Research Society, Tallinn, Estonia)

F. Wehrle & B. Latal (June 26th 2014): *Schulprobleme von Frühgeborenen: Sind es die exekutiven Funktionen? (*Tagung der Abteilung Entwicklungspädiatrie, University Children's Hospital Zurich)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (June 12th - 13th 2014): *Impaired Executive Functions in Complex Tasks in Children and Adolescents Born Very Preterm* (3. Gemeinsamer Jahreskongress von SGP, SGKC und SKGJPP, Basel, Switzerland)

2013 **F. Wehrle**, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (September 3rd - 7th 2013): *Multimodal Approach to Neurocognitive Deficits in Children and Adolescents Born Preterm* (16th European Conference on Developmental Psychology, Lausanne, Switzerland)

Poster presentation

2016 R. O'Gorman, **F. Wehrle**, T. Wood, A. Buchmann, B. Latal, R. Huber, S. Deoni, G. Barker, & C. Hagmann (April 30th - May 3rd 2016): Quantitative T1 relaxation times in very preterm children and adolescents (Pediatric Academic Societies (PAS) Annual Meeting, Baltimore)

2015 **F. Wehrle**, B. Latal, R. O'Gorman, C. Hagmann, & R. Huber (September 15th - 20th 2015): *EEG Sleep Slow Wave Activity as a Potential Marker of Load-Dependent Deficits in Executive Functions in Very Preterm Children and Adolescents* (1st Congress of Joint European Neonatal Societies, Budapest, Hungary)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, C. Hagmann, & R. Huber (June 11th - 12th 2015). Altered Topography of Sleep Spindle Activity in Very Preterm Children and Adolescents (Gemeinsame Jahresversammlung of the Swiss Society of Paediatrics (SGP) and the Swiss Society for Sleep Research, Sleep Medicine and Chronobiology (SSSSC), Interlaken, Switzerland)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, C. Hagmann, & R. Huber (April 25th - 28th 2015): *Working Memory Ability and Topographical Distribution of Sleep Slow Wave Activity in Children and Adolescents* (Pediatric Academic Societies (PAS) Annual Meeting, San Diego)

2014 **F. Wehrle,** A. Buchmann, A. Hüsser, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (October 17th - 21st 2014): *Volume of Cerebellum and Thalamus Is Associated with Working Memory Performance in Children and Adolescents Born Very Preterm* (5th Congress of the European Academy of Paediatric Societies, Barcelona, Spain)

F. Wehrle, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (May 15th - 17th 2014): Impaired Executive Functions in Complex Tasks in Children and Adolescents Born Very Preterm (Inspiring Infancy Meeting, Groningen, the Netherlands)

S. Amin, **F. Wehrle**, R. Huber, B. Latal, R. O'Gorman, H. Speckbacher, & C. Hagmann (May 3rd - 6th 2014): White Matter Microstructural Differences Between Prematurely Born Adolescents Relative to Term Born Controls Using TBSS (Pediatric Academic Societies (PAS) Annual Meeting, Vancouver, Canada)

2013 **F. Wehrle**, B. Latal, L. Kaufmann, R. O'Gorman, R. Huber, & C. Hagmann (October 31st 2013): Executive Functions in School-Aged Children and Adolescents Born Very Preterm (Children's Research Center (CRC) retreat, University Children's Hospital Zurich)

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